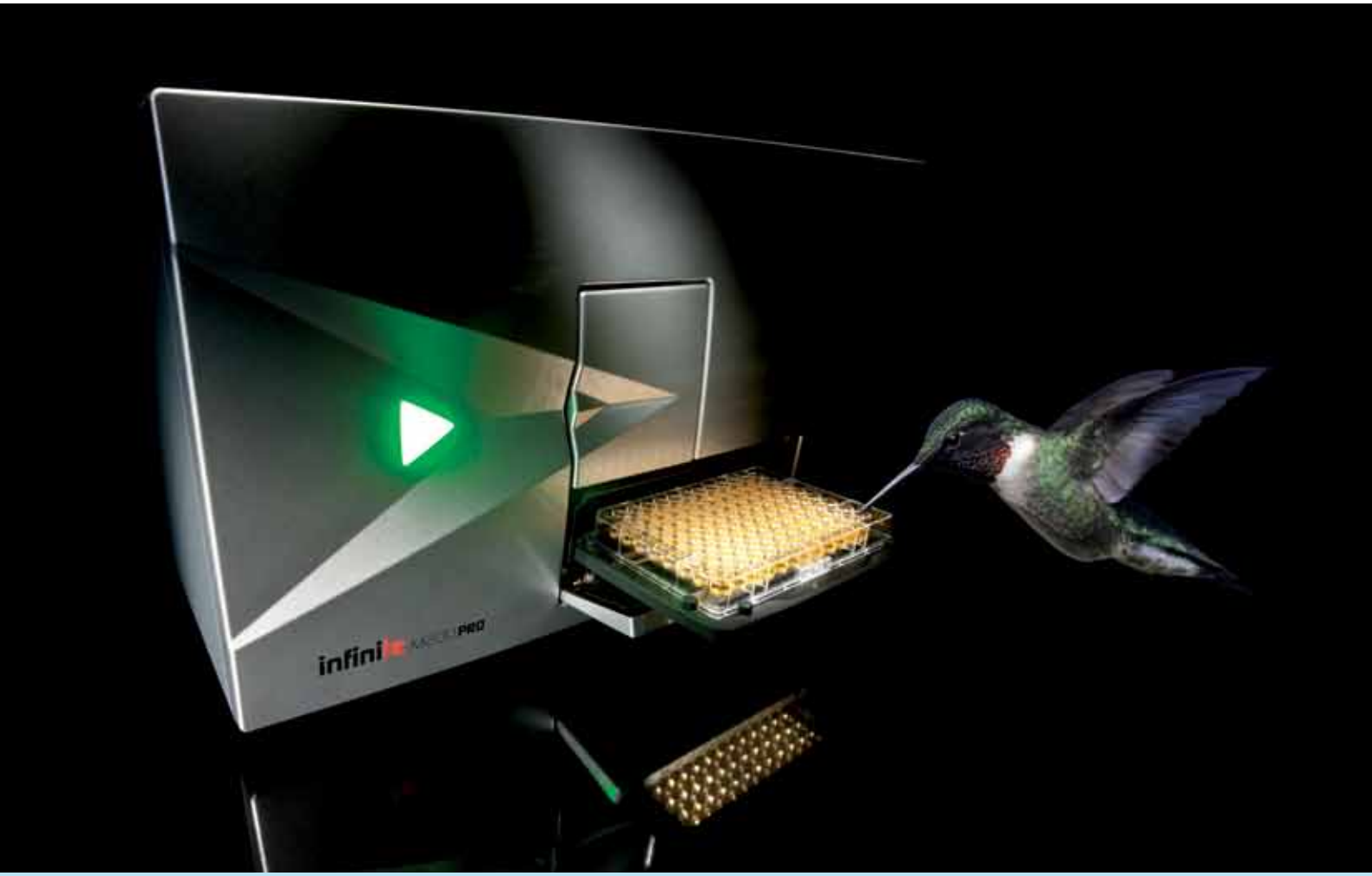
TECAN.

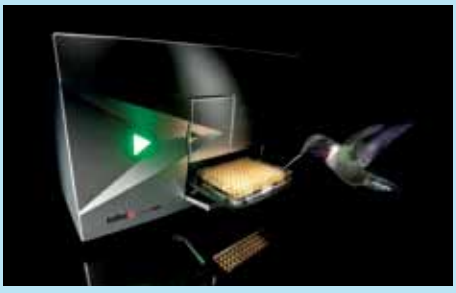


Application Guide for Multimode Readers

Your easiest way to optimal results!

DLReady™ LanthaScreen® Certified® invitrogen TRANSCREENER® For Red FP validated TRANSCREENER® Red FI validated TRANSCREENER® Red TR-FRET validated htrf This reader is HTRF® compatible quad4 monochromators™ premium quad4 monochromators™ 2µl NanoQuant Plate™ compatible

Technology Orientation



Tecan's multimode readers are symbolized by a hummingbird, the embodiment of speed, precision, and color. Like the plumage of the hummingbird, our monochromators cover the entire spectrum of the visible light. Hummingbirds can hover in mid-air to target flowers even smaller than a well of a 96-well plate, mirroring the precision of instrument measurements. The hummingbird's heart rate, which can reach as high as 1,260 beats per minute to support the rapid beating of its wings of up to 80 times per second, represents the exceptional speed and throughput rate of Tecan's multimode readers.

Table of content (alphabetical)

ABS – Absorbance

Light is absorbed by the sample

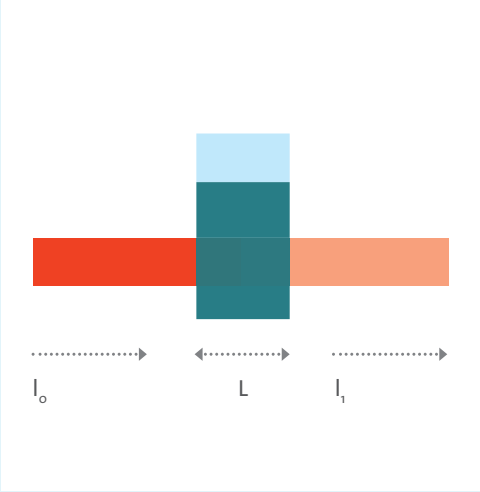


Figure 1: Schematic representation of an absorbance measurement performed in a cuvette. I_0, I_1: Intensity of light before (I_0) and after (I_1) passing the cuvette with length L.

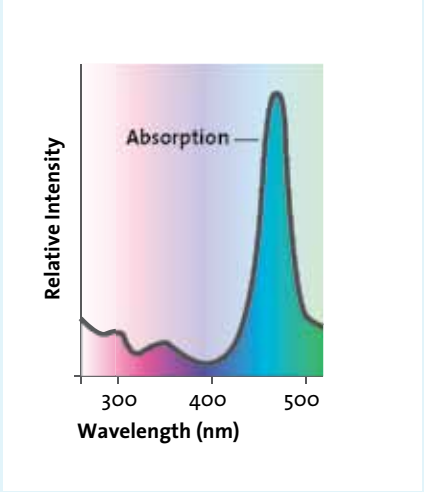


Figure 2: An absorbance spectrum shows the extent of light absorption at any specific wavelength.

Absorbance	Transmittance [%]
0	100
1	10
2	1
3	0.1

Table 1: The relationship between absorbance and transmittance values. An absorbance value of 3 means that only 0.1 % of the light is able to pass through the sample. Most multimode readers can only read samples up to an OD of 4.

Technology

When light shines through a turbid or colored liquid, some of its intensity is absorbed by the liquid's molecules or particles (Figure 1). The amount of light that penetrates the sample and reaches the detector is called the transmittance (T), and the light absorbed by the sample is called the absorbance (A, Abs), or optical density (OD)[1].

OD values commonly correspond to a 1 cm path length, which is the width of standard cuvettes (Figure 1). Note that absorbance is a logarithmic function of the transmittance (Table 1), as defined by the Beer-Lambert law[2]:

$$A = -\log_{10}(I_1/I_0)$$

The absorbance spectrum is a function of the molecule; light of different wavelengths can be absorbed differently depending on the sample, as shown in the absorbance spectrum in Figure 2. For this reason, the absorbance is always stated together with the wavelength, for example OD_{600}.

Major applications

- DNA / RNA quantification (Abs)
- MTT / MTS assays
- BCA, Modified Lowry and Bradford assays – Protein quantification

[1] Bioanalytik. Von F. Lottspeich. Spektrum, Heidelberg, 1998
[2] Beer (1852) "Bestimmung der Absorption des rothen Lichts in farbigen Flüssigkeiten" (Determination of the absorption of red light in colored liquids), Annalen der Physik und Chemie, vol. 86, pp. 78–88.

Compatible readers




Features	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Reader design	Monochromator	Filter	Filter	Monochromator
Configuration	Quad4 Monochromators™, 2 excitation and 2 emission monochromators for high performance, high flexibility and accurate data acquisition	Up to 4 programmable filter pairs per slide that are easily ejected and exchanged through the front of the instrument	Up to 6 filter pairs per slide which are individually moveable, allowing any combination of Ex and Em filters; easy filter change, ID chip	Premium Quad4 Monochromators, 2 excitation and 2 emission monochromators allow for stray light reduction up to a factor of 10^7
Capability scanning	Yes	No	No	Yes
Fastest read time	20 sec (96)	20 sec (96)	14 sec (96)	11 sec (96)
Plate format	6- to 384-well plates NanoQuant Plate Cuvette port	6- to 384-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate
Temp. control	Ambient +5 °C to 42 °C	Ambient +5 °C to 42 °C	Ambient +4 °C to 42 °C	Ambient +4 °C to 42 °C
Shaking	Linear, orbital with variable amplitudes	Linear, orbital with variable amplitudes	Linear, orbital with variable amplitudes	Linear, orbital, double orbital with variable amplitudes
Injectors	2	2	2	2
Gas Control Module	CO_2 and O_2	CO_2 and O_2	-	-
Stacker	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	On-board stacker: stacks for 30 or 50 plates
Barcode reader	Yes	Yes	Yes	Yes
Cuvette port	Yes	No	No	No

Typcal values: Absorbance	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Detector	UV silicon photodiode	UV silicon photodiode	UV silicon photodiode	UV silicon photodiode, 4-channel parallel reading
Light source	UV xenon flash lamp	UV xenon flash lamp	High energy xenon flash lamp	High energy xenon flash lamp
Wavelength selection	Monochromator	Filter	Filter	Monochromator
Wavelength range	230 - 1000 nm	230 - 1000 nm	230 - 1000 nm	230 - 1000 nm
Dynamic range	0 - 4 OD	0 - 4 OD	0 - 4 OD	0 - 4 OD
Bandwith	<5 nm for λ ≤315 nm; <9 nm for λ >315	Filter dependent	Filter dependent	Fixed, 5 nm
Accuracy	0 - 2 OD: ≤ ± (1 % + 10 mOD) 2 - 3 OD: ≤ ± 2.5 %	0 - 2 OD: ≤ ± (1 % + 10 mOD) 2 - 3 OD: ≤ ± 2.5 %	0 - 2 OD: < ±0.8 % + 8 mOD	0 - 3 OD: ≤ ± (1 % + 6 mOD)
Precision	< 0.2 % @ 260 nm	Filter dependent	Filter dependent	0 - 3 OD: ≤ ± (0.5 % + 5 mOD)
Linearity	R^2 = 0.999 (0 - 2 OD)	R^2 = 0.999 (0 - 2 OD)	R^2 = 0.999 (0 - 2 OD)	0 - 3 OD: R^2 ≥0.999
Baseline flatness	±10 mOD (1 sigma)	±10 mOD (1 sigma)	±10 mOD (1 sigma)	±10 mOD (1 sigma)
Stray light	Reduction by a factor of 10^6			Reduction by a factor of 10^7

Support

Tecan Application Notes

- Automated solution for monitoring growth of Staphylococcus aureus
- Explore the world in tiny drops
- Cell Proliferation and Cell Viability Analysis in in vitro Systems-Cell Culture Methods on Tecan's Infinite® 200
- NanoQuant Plate – Low Volume DNA Quantification for Affymetrix® GeneChip
- pION Assay - Development of PAMPA model for skin penetration of drugs
- RNA quantification: Sorted Mouse Keratinocyte Stem Cells at Karolinska institute

DNA / RNA quantification (Abs)

DNA / RNA quantification based on absorbance

Figure 2: Tecan NanoQuant Plate™

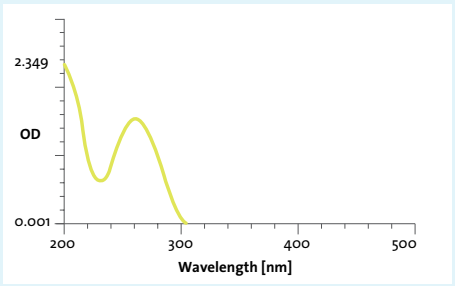


Figure 1: DNA absorbance spectum.

$1OD_{260}$	Concentration (µg/ml)
dsDNA	50
ssDNA	33
RNA	40
Oligonucleotides	20–30

Table 1: Nucleic acid concentrations at OD_{260} = 1 and neutral pH

Assay overview

Technology
Absorbance

DNA and RNA can be quantified based on absorbance at 260 nm, which is in the UV range and not visible to the human eye (Figure 1). Tecan's multimode readers provide cuvette ports for DNA and RNA measurement (quartz or UV-transparent cuvettes must be used). Alternatively, Tecan's patented NanoQuant Plate™ (Figure 2) is ideal for smaller volumes (2 µl), higher throughput (16 samples at once), and more economical DNA / RNA quantification.
1 OD_{260} corresponds to different concentrations, depending on the type of nucleic acid being quantified (Table 1).

The ratio of absorption at 260 nm vs 280 nm is commonly used to assess DNA contamination of protein solutions, since proteins – in particular, the aromatic amino acids – absorb light at 280 nm[3,4]. It is generally acknowledged that pure DNA has a ratio of 2, and RNA, 1.8[5]. Generally the A_{260}/A_{230} ratio also provides valuable information about the nucleic acid purity[6].

Typical detection limits for absorbance based DNA quantification are within the ng/µl range.

Alternative
LifeTechnologies' PicoGreen and RiboGreen quantification assays provide a broader dynamic range.

Sample protocol for DNA measurements
1. Blank the reader with the same buffer used to dilute the DNA
2. Take an appropriate volume of sample (NanoQuant Plate: 2µl, cuvettes: volume depends on the min. / max. filling volume)
3. Measure OD_{260} and OD_{280}
4. If the OD_{260} value is greater than two, dilute samples
5. To calculate the concentration, multiply the OD_{260} by the concentration factor shown in Table 2 and your dilution factor, if applicable
6. Determine the purity by dividing the value for OD_{260} by the value for OD_{280}

[3] a b c d e Sambrook and Russell (2001). Molecular Cloning: A Laboratory Manual (3rd ed.). Cold Spring Harbor Laboratory Press. ISBN 978-0-87969-577-4.
[4] (Sambrook and Russell cites the original paper: Warburg, O. and Christian W. (1942). "Isolierung und Kristallisation des Gärungsferments Enolase". Biochem. Z. 310: 384–421.)
[5] Glasel, J.A. (1995) Validity of Nucleic Acid Purities Monitored by A260/A280 Absorbance Ratios, Biotechniques 18:62-63
[6] http://www.qiagen.com/literature/benchguide/default.aspx

Instrument parameters




Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**DNA concentration**	**DNA concentration**	**DNA concentration**	**DNA concentration**
Wavelength	260	260	260	260
Bandwith	5 nm	5 nm	5 nm	5 nm
Flashes	25	25	10	10
Settle time	0 ms	0 ms	0 ms	0 ms
Shaking	-	-	-	
Assay	**Purity check**	**Purity check**	**Purity check**	**Purity check**
Wavelength	260/280	260/280	260/280	260/280
Bandwith	5 nm	5 nm	5 nm	5 nm
Flashes	25	25	10	10
Settle time	0 ms	0 ms	0 ms	0 ms
Shaking	0 sec.	0 sec.	0 sec.	0 sec.

Support

Link

- Qiagen general DNA/RNA purification protocols: http://www.qiagen.com/literature/benchguide/default.aspx

Tecan Application Notes

- RNA quantification: Sorted Mouse Keratinocyte Stem Cells at Karolinska institute
- Low Volume DNA and RNA Quantification – NanoQuant
- NanoQuant Plate – Low Volume DNA Quantification for Affymetrix® GeneChip
- Explore the world in tiny drops

MTT[7]/MTS[8] assay

Cell viability / cytotoxicity assays with absorbance readout

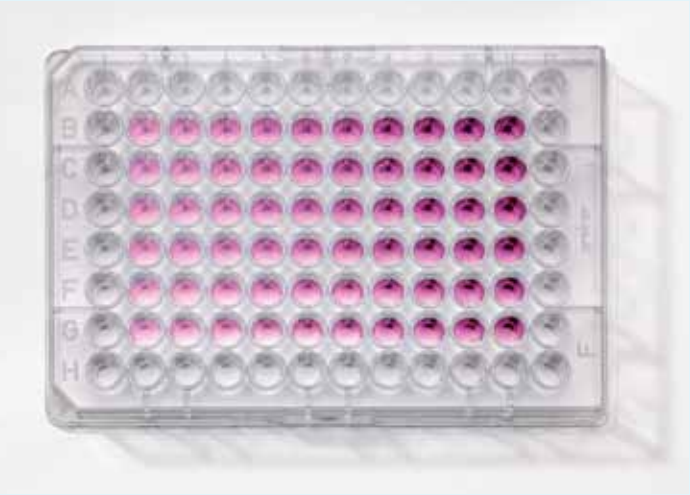


Figure 1: MTT assay in a 96-well format

Assay overview

Technology
Absorbance

Principle, Major application
The MTT and MTS assays are colorimetric (Figure 1) – and hence absorbance – assays that measure cell viability. MTT / MTS is taken up by the cells and processed to a varying extent depending on the cell viability. Viable cells reduce more MTS / MTT to formazan, yielding a more intense purple color.

Mechanism
While MTT assays need to use a reagent to make the formazan generated soluble, MTS assays yield water-soluble products, potentially making them homogeneous assays.

MTT assays use a solubilization reagent, such as dimethyl sulfoxide (DMSO) or isopropanol, to dissolve the non-water-soluble formazan product, yielding a colored solution that can be quantified by absorbance measurement at approximately 565 nm, dependent on the solvent employed[9].

The MTS assay is an improved version of the MTT assay. Its reagents are reduced more efficiently within the cell than MTT, and the resulting product is water-soluble and less cytotoxic than the insoluble formazan used in the MTT assay. This makes it a one-step (homogeneous) assay, with the convenience of adding the reagent directly to the cell culture without the intermittent steps required in the MTT assay.

However, when MTS is used in a homogeneous way the assay becomes susceptible to colorimetric interference, as traces of colored compounds may remain in the microplate[10].

Provider
MTT, MTS and similar reagents are available from various chemistry distributors, or as the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega)[11].

Alternatives
PrestoBlue® Cell Viability Reagent[12], a new resazurin-based development from Invitrogen that offers a much shorter incubation time. Besides that, also fluorescence-based or time-resolved fluorescence assays are available.

[7] MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-phenylditetrazolium bromide, a yellow tetrazole)
[8] MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)
[9] Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods 65 (1-2): 55–63.
[10] Cory AH, Owen TC, Barltrop JA, Cory JG (1991). Use of an aqueous soluble tetrazolium/formazan assay for cellgrowth assays in culture. Cancer communications 3 (7): 207–12.
[11] http://www.promega.com/products/cell-health-assays/cell-viability-assays/celltiter-96-aqueous-one-solution-cellproliferation-assay-_mts_/
[12] http://www.invitrogen.com/site/us/en/home/brands/Molecular-Probes/Key-Molecular-Probes-Products/PrestoBlue-Cell-Viability-Reagent.html

Instrument parameters




Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**MTT assay CellTiter 96 AQueous One**	**MTT assay CellTiter 96 AQueous One**	**MTT assay CellTiter 96 AQueous One**	**MTT assay CellTiter 96 AQueous One**
Wavelength	565 nm	565 nm	565 nm	565 nm
Bandwith	9 nm	10 nm	10 nm	10 nm
Flashes	25	25	10	10
Settle time	0 ms	0 ms	0 ms	0 ms
Shaking	180 sec; 1 mm amplitude; orbital	180 sec; 1 mm amplitude; orbital	180 sec; 1 mm amplitude; orbital	180 sec; 1 mm amplitude; orbital

Support

Link

- Promega – CellTiter 96 AQueous One Solution Cell Proliferation Assay: http://www.promega.com/products/cell-health-assays/cell-viabilityassays/celltiter-96-aqueous-one-solution-cell-proliferation-assay-_mts_/
- PrestoBlue® Cell Viability Reagent: http://www.invitrogen.com/site/us/en/home/brands/Molecular-Probes/Key-Molecular-Probes-Products/PrestoBlue-Cell-Viability-Reagent.html

Tecan Application Notes

- Cell Proliferation and Cell Viability Analysis in in vitro Systems- Cell Culture Methods on Tecan´s Infinite® 200
- LIVE/DEAD Viability / Cytotoxicity assay
- Detection of Calcein-AM and Hoechst 33342

BCA, Modified Lowry and Bradford assays – Protein quantification

Protein quantification assays with absorbance readout

Figure 1: Bradford Protein Assay measured in cuvettes, showing increasing protein concentrations.

Assay overview

Technology
Absorbance

Principle, Major application
All three assays are designed to determine the protein concentration of a sample. For detection, a liquid reagent needs to be added to the samples. This reagent interacts with the proteins, leading to a visible color change (Figure 1) that is directly proportional to the protein concentration. Absolute concentrations are calculated using a standard curve.

Provider
Various companies have established their own assays for this purpose. The main differences between the various assays are the dynamic range and the measurement wavelength.

Mechanism
The **BCA™** Protein Assay (Thermo Scientific Pierce) uses bicinchoninic acid (BCA) for colorimetric quantification of total protein in a sample[13]. The method is based on the reduction of Cu^{2+} to Cu^{+} by proteins in an alkaline medium to form a colored watersoluble chelate that can be measured at its absorption maximum of 562 nm. The linear working range for BSA is 20 to 2000 µg/ml[14].
The **Bradford** Protein Assay (BioRad) is based on the Coomassie® Brilliant Blue G-250 dye which binds to basic and aromatic amino acid residues, particularly arginine. This induces a shift of the dye's absorbance maximum from 465 nm to 595 nm. The Bradford assay can be performed as a microassay procedure, with a linearity range of 125 to 1,000 µg/ml BSA[15].
In the **Modified Lowry** Protein Assay (Thermo Scientific Pierce), the protein reacts with cupric sulfate and tartrate in an alkaline solution, which results in formation of a tetradentate copper-protein complex, reducing the Folin-Ciocalteu Reagent. The absorbance of the blue, water-soluble product can be measured at 750 nm. The assay – tested with BSA protein[16] – exhibits good linearity in the range of 1 to 1500 µg/ml.

Alternatives
Potential alternatives for protein quantification range from absorbance-based methods using the protein extinction coefficient[17] to fluorescence based assays like NanoOrange® to even dedicated[18] devices.

[13] Smith, P.K., et al.: Measurement of protein using bicinchoic acid. Anal Biochem., 150, 76-85, 1985
[14] http://www.piercenet.com/browse.cfm?fldID=02020101
[15] http://www.bio-rad.com/webroot/web/pdf/lsr/literature/4110065A.pdf
[16] http://www.piercenet.com/browse.cfm?fldID=02020103
[17] http://web.expasy.org/protparam/protparam-doc.html
[18] http://www.millipore.com/techpublications/tech1/an2222en

Instrument parameters




Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**BCA assay**	**BCA assay**	**BCA assay**	**BCA assay**
Wavelength	565 nm	562 nm	562 nm	565 nm
Bandwith	9 nm	10 nm	10 nm	9 nm
Flashes	25	25	10	10
Settle time	0 ms	0 ms	0 ms	0 ms
Shaking	0 sec.	0 sec.	0 sec.	0 sec.
Assay	**Modified Lowry**	**Modified Lowry**	**Modified Lowry**	**Modified Lowry**
Wavelength	750 nm	750 nm	750 nm	750 nm
Bandwith	9 nm	10 nm	10 nm	9 nm
Flashes	25	25	10	10
Settle time	0 ms	0 ms	0 ms	0 ms
Shaking	0 sec.	0 sec.	0 sec.	0 sec.
Assay	**Bradford**	**Bradford**	**Bradford**	**Bradford**
Wavelength	595 nm	590 nm	590 nm	595 nm
Bandwith	9 nm	10 nm	10 nm	9 nm
Flashes	25	25	10	10
Settle time	0 ms	0 ms	0 ms	0 ms
Shaking	0 sec.	0 sec.	0 sec.	0 sec.

Support

Links

- BCA assay: http://www.piercenet.com/browse.cfm?fldID=02020103
- Modified Lowry assay: http://www.piercenet.com/browse.cfm?fldID=02020103
- Bradford assay homepage: http://www.bio-rad.com/prd/en/US/adirect/biorad?ts=1&cmd=BRCatgProductDetail&vertical=LSR&catID=d4d4169a-12e8-4819-8b3e-ccab019c6e13
- NanoOrange: http://products.invitrogen.com/ivgn/product/N6666

Tecan Application Notes

- Protein Quantification in Small-Volume Samples
- Protein quantification: BCA™, Modified Lowry and Bradford assays
- Protein quantification on Infinite™ 200 with injectors

FI – Fluorescence Intensity

Light is absorbed and released (emitted)

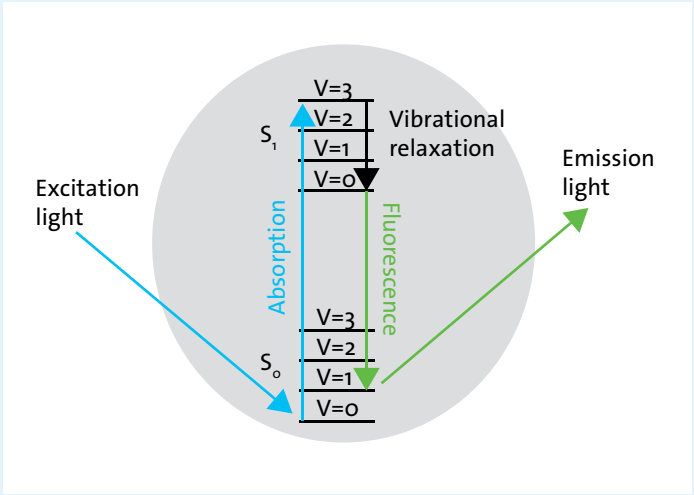


Figure 1: Jablonski diagram drawn inside a fluorescence molecule. S = electronic state, V = vibrational level. After photon absorption (= excitation), the molecule adopts a state of higher energy S_1 (= excited state) including several vibrationally excited substates. By vibrational relaxation, the molecule relaxes to the lowest excited S_1 state (black arrow). From this state the molecule relaxes into the vibrational states of S_0 by emitting light.

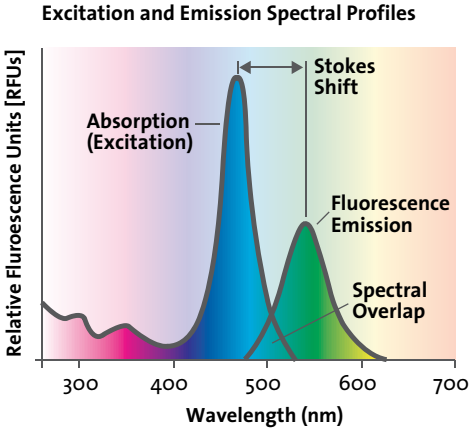


Figure 2: While the excitation spectrum describes how efficient it is to excite the fluorophore at a specific wavelength, the emission spectrum describes how efficient it is to detect the emitted light at any given wavelength. The Stokes shift describes the distance between the excitation and emission maximum, and is given in nanometers (nm).

Technology

Fluorescence describes a molecule's ability to emit (release) previously absorbed light (Figure 1). The emission occurs almost instantly (within 1 nano second) and, according to the laws of physics, the emitted light will always have a higher wavelength and hence a lower energy.

A fluorescence spectrum consists of an absorption (excitation) and emission spectrum (Figure 2). Fluorescence labels (fluorophores) can be attached to any available biomolecule and used to answer quantitative, as well as qualitative, questions. For example, 'does the sample contain the fluorophore?' (qualitative), and 'how much of the fluorophore is in the sample?' (quantitative). Signals are quantified as Relative Fluorescence Units [RFU].

Major applications

- PicoGreen® and RiboGreen® DNA / RNA quantification
- Resazurin assay
- GFP (Green Fluorescent Protein)

Support

Link

- General explanation of fluorescence: http://www.olympusmicro.com/primer/lightandcolor/fluorointroduction.html

Tecan Application Notes

- Tweaking fluorescence scans
- Optimizing the acquisition of 3D fluorescence spectra
- Analyzing biological drug effects in 3D
- Human TNF-α ELISA using Sword™ Peroxidase Reagents
- Peroxidase detection using Sword™ Peroxidase Reagents
- Human IL-6 chemiluminescent ELISA using Sword™ Peroxidase Reagents
- Human C-reactive protein ELISA using Sword™ Peroxidase Reagents
- Fluorescence-Based DNA Quantification in Small Volume Samples
- DNA and RNA quantification: fast and simple with PicoGreen® dsDNA and RiboGreen® RNA quantification reagents

Compatible readers




Features	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Reader design	Monochromator	Filter	Filter	Monochromator
Configuration	Quad4 Monochromators™, 2 excitation and 2 emission monochromators for high performance, high flexibility and accurate data acquisition	Up to 4 programmable filter pairs per slide that are easily ejected and exchanged through the front of the instrument	Up to 6 filter pairs per slide which are individually moveable, allowing any combination of Ex and Em filters; easy filter change, ID chip	Premium Quad4 Monochromators, 2 excitation and 2 emission monochromators allow for stray light reduction up to a factor of 10^7
Transcreener FI	Yes	Yes	Yes	Yes
Capability – scanning	Yes	No	No	Yes
Capability – scanning 3D	No	No	No	Yes
Dichroic mirrors	-	Dichroic mirror, optimized for TRF measurements (Eu, Tb, Sm)	Multiple dichroic mirrors	-
Z-adjustment	Automatic	-	Automatic	Automatic
Plate format	6- to 384-well plates NanoQuant Plate	6- to 384-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate
Temperature control	Ambient +5 °C to 42 °C	Ambient +5 °C to 42 °C	Ambient +4 °C to 42 °C	Ambient +4 °C to 42 °C
Shaking	Linear, orbital with variable amplitudes	Linear, orbital with variable amplitudes	Linear, orbital with variable amplitudes	Linear, orbital, double orbital with variable amplitudes
Injectors	2	2	2	2
Stacker	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	On-board stacker: stacks for 30 or 50 plates
Barcode reader	Yes	Yes	Yes	Yes

Typcal values: Fluorescence intensity (FI)	**Infinite M200 PRO**	**Infinite F200 PRO**	**Infinite F500**	**Infinite M1000 PRO**
Capability top	Yes	Yes	Yes	Yes
Capability bottom	Yes	Yes	Yes	Yes
Light source	UV xenon flash lamp	UV xenon flash lamp	High energy xenon flash lamp	High energy xenon flash lamp
Detector	PMT, optionally UV and red-sensitive	PMT, optionally UV and red-sensitive	Extended wavelength (UV and far-red) Low dark current PMT	Extended wavelength (UV and far-red) Low dark current PMT
Wavelength range	Standard: Ex: 230 - 600 nm Em: 330 - 600 nm Optional: Ex: 230 - 850 nm Em: 280 - 850 nm	Standard: Ex: 230 - 600 nm Em: 330 - 600 nm Optional: Ex: 230 - 850 nm Em: 280 - 850 nm	Ex: 230 - 900 nm Em: 280 - 900 nm	Ex: 230 - 850 nm Em: 280 - 850 nm
Sensitivity – top	170 amol / well (1.7pM; 384-well plate)	85 amol / well (0.85pM, 384-well plate)	10 amol / well fluorescein (Greiner® 384-well low volume black plate; 10 µl), 1pM	25 amol / well fluorescein (Greiner 384-well low volume black plate; 10 µl), 2.5pM
Sensitivity – bottom	1.2 fmol / well (6pM; 96-well plate)	0.7 fmol / well (3.5pM; 96-well plate)	0.4 fmol / well fluorescein (Greiner 96-well SensoPlate™; 200 µl), 2pM	0.6 fmol / well fluorescein (Greiner 96-well SensoPlate; 200 µl), 3pM
Wavelength accuracy	< ±2 nm for λ >315 nm; < ±1 nm for λ ≤315 nm	Filter dependent	Filter dependent	≤300 nm: ±0.5 nm; >300 nm: ±1 nm
Wavelength reproducibility	< ±1 nm for λ >315 nm; < ±0.5 nm for λ ≤315 nm	Filter dependent	Filter dependent	≤300 nm: ±0.5 nm; >300 nm: ±1 nm
Bandwidth	Ex: <5 nm for λ ≤315 nm and <9 nm for λ >315 Em: <10 nm for λ ≤315 nm and <20 nm for λ >315	Filter dependent	Filter dependent	Adjustable ≤300 nm: 2.5 - 10 nm; >300 nm: 5 - 20 nm
Wavelength selection	Monochromator	Filter	Filter	Monochromator

PicoGreen® and RiboGreen® DNA / RNA quantification

High sensitivity, fluorescence-based DNA / RNA quantification

Figure 1: Green DNA

Assay overview

Technology
Fluorescence Intensity

Assay design and provider
LifeTechnologies' PicoGreen[19] and RiboGreen[20] (Figure 1) quantification assays use a fluorescence approach to determine DNA and RNA concentrations. Using the Quant-iT PicoGreen dsDNA Assay Kit, you can selectively detect as little as 25 pg/ml of dsDNA in the presence of ssDNA, RNA, and free nucleotides. The assay is linear over three orders of magnitude, and has little sequence dependence, allowing you to accurately measure DNA from many sources.

RiboGreen RNA reagent is one of the most sensitive detection dyes for the quantification of RNA in solution, offering linear fluorescence detection in the range of 1 to 200 ng/ml of RNA.

Mechanism
Both assays are easy to use; simply add the dye to the sample, wait five minutes, and then read.

Alternatives
If sensitivity is not a major issue, it is possible to perform DNA quantification using absorbance at 260 nm.

[19] http://products.invitrogen.com/ivgn/product/P7589
[20] http://products.invitrogen.com/ivgn/product/R11490

Instrument parameters




Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**PicoGreen, RiboGreen**	**PicoGreen, RiboGreen**	**PicoGreen, RiboGreen**	**PicoGreen, RiboGreen**
Mode	Top	Top	Top	Top
Excitation wavelength	485 nm (9 nm)	485 nm (20 nm)	485 nm (20 nm)	485 nm (9 nm)
Emission wavelength	535 nm (20 nm)	535 nm (25 nm)	535 nm (25 nm)	535 nm (20 nm)
Lag time	0 µs	0 µs	0 µs	0 µs
Integration time	20 µs	20 µs	20 µs	20 µs
Flashes	25	25	10	10
Mirror	automatic	automatic	automatic	automatic
Gain	optimal	optimal	optimal	optimal
Z-position	automatic	/	automatic	automatic
Settle time	0 ms	0 ms	0 ms	0 ms

Support

Links

- Quant-iT™ RiboGreen RNA Assay Kit: http://products.invitrogen.com/ivgn/product/R11490
- Quant-iT PicoGreen dsDNA Assay Kit: http://products.invitrogen.com/ivgn/product/P7589

Tecan Application Notes

- Fluorescence-Based DNA Quantification in Small Volume Samples
- DNA and RNA quantification: fast and simple with PicoGreen® dsDNA and RiboGreen® RNA quantification reagents
- PicoGreen® assay measured in NanoQuant Plate™
- Impact of Extended Adjustable Monochromator Bandwidth in Fluorescence Based Application Technologies
- Comparison of two different detection techniques for DNA

Resazurin assay

A Fluorescence Intensity-based (bacterial / cell) proliferation assay

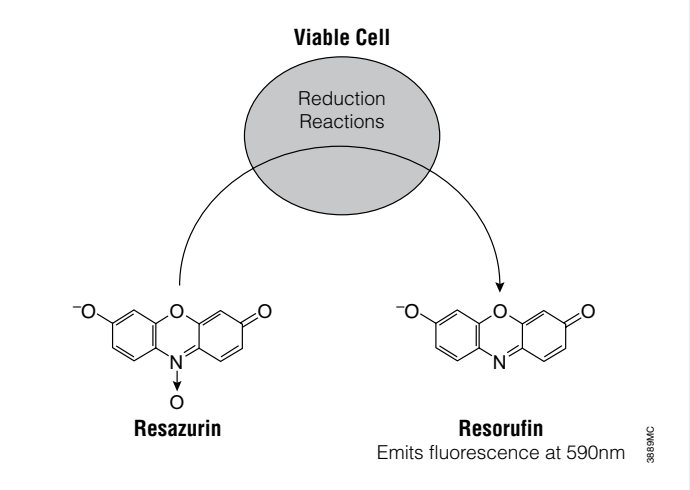


Figure 1: Viability dependet conversion of Resazurin to Resorufin (Promega)

Assay overview

Technology
Fluorescence Intensity

Major application
Cell viability assays

Provider
Resazurin was initially used for bacterial studies, but is now also available for eukaryotic cell-based applications under brand names such as the alamarBlue® assay[21] (Life Technologies) and CellTiter-Blue® Cell Viability Assay[22] (Promega).

Mechanism
Resazurin is a redox indicator that can be added directly to cells. Viable cells convert the dark blue, oxidized form of the dye (resazurin) into a red, fluorescent reduced form called resorufin (Ex: 570 nm; Em: 590 nm). The amount of fluorescence (or absorbance) is proportional to the number of living cells, and corresponds to the cell's metabolic activity. Damaged and non-viable cells have lower innate metabolic activity, and therefore generate a proportionally lower signal than healthy cells. The system is specific for cell viability as non-viable cells rapidly lose metabolic capacity and do not reduce resazurin. Consequently, a fluorescent signal[23] is not generated.

Alternatives
PrestoBlue Cell Viability Reagent[24], a new development from Invitrogen that offers much shorter incubation times. The absorbance based MTT/MTS assay.

[21] http://www.invitrogen.com/site/us/en/home/brands/Molecular-Probes/Key-Molecular-Probes-Products/alamarBlue-Rapid-and-Accurate-Cell-Health-Indicator.html
[22] http://www.promega.com/products/cell-health-assays/cell-viability-assays/celltiter_blue-cell-viability-assay/
[23] O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur. J. Biochem. 2000, 267,5421–5426.
[24] http://www.invitrogen.com/site/us/en/home/brands/Molecular-Probes/Key-Molecular-Probes-Products/PrestoBlue-Cell-Viability-Reagent.html

Instrument parameters




Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**alamarBlue CellTiter-Blue**	**alamarBlue CellTiter-Blue**	**alamarBlue CellTiter-Blue**	**alamarBlue CellTiter-Blue**
Mode	Bottom	Bottom	Bottom	Bottom
Excitation wavelength	560 nm (9 nm)	560 nm (10 nm)	560 nm (10 nm)	560 nm (10 nm)
Emission wavelength	600 nm (20 nm)	600 nm (20 nm)	600 nm (20 nm)	600 nm (20 nm)
Lag time	0 µs	0 µs	0 µs	0 µs
Integration time	20 µs	20 µs	20 µs	20 µs
Flashes	25	25	10	10
Mirror	/	automatic	automatic	/
Gain	optimal	optimal	optimal	optimal
Z-position	automatic	/	automatic	automatic
Settle time	0 ms	0 ms	0 ms	0 ms

Support

Links

- Promega CellTiter-Blue Cell Viability Assay: http://www.promega.com/products/cell-health-assays/cell-viability-assays/celltiter_blue-cell-viability-assay/
- Invitrogen alamarBlue assay: http://www.invitrogen.com/site/us/en/home/brands/Molecular-Probes/Key-Molecular-Probes-Products/alamarBlue-Rapid-and-Accurate-Cell-Health-Indicator.html
- PrestoBlue Cell Viability Reagent: http://www.invitrogen.com/site/us/en/home/brands/Molecular-Probes/Key-Molecular-Probes-Products/PrestoBlue-Cell-Viability-Reagent.html

Tecan Application Notes

- Cell Proliferation and Cell Viability Analysis in in vitro Systems- Cell Culture Methods on Tecan´s Infinite® 200
- LIVE/DEAD Viability / Cytotoxicity assay
- Detection of Calcein-AM and Hoechst 33342

GFP (Green Fluorescent Protein)

Fluorescent protein frequently used as an expression / activation reporter

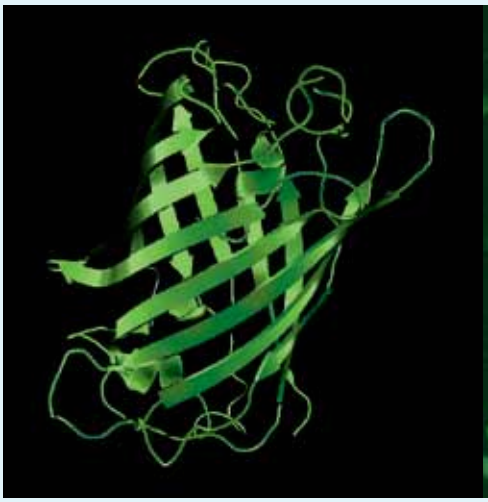


Figure 1: Protein structure of GFP

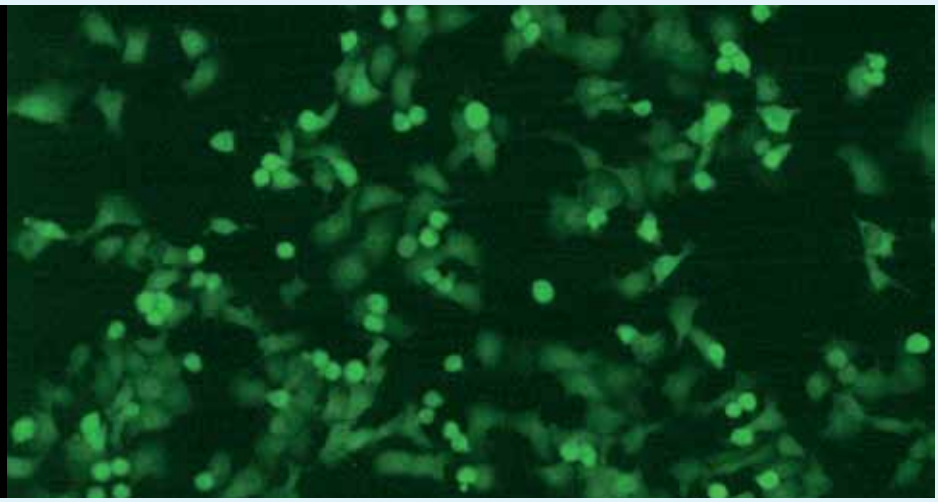


Figure 2: GFP-transfected eukaryotic cells

Assay overview

Technology
Fluorescence Intensity

Principle
GFP (Figure 1) is a protein derived from a jellyfish which has the ability to emit light in the green wavelength range and can be detected using standard FI measurements.

Major applications
GFP can be used in multiple different of ways, for example as a BRET / FRET partner in binding studies, or for gene activation, where it is often fused / cloned to a gene of interest and co–expressed once the gene is activated (Figure 2). Commonly, it is differentiated between constitutive (permanent) and temporary expression. Constitutive expression is mostly used to monitor growth or proliferation of cells or bacteria, while temporary expression is used for gene activation studies.

Format, provider
Due to multiple engineering efforts, an almost unlimited number of mutants exist, resulting in a large bandwidth of excitation and emission values. Some of these variants are commercially available, while others are published and therefore not protected. Consequently, only a selection of measurement parameters can be given, since the wavelength depends on the mutant type of the protein.

Alternatives
Technology-wise, there are a lot of alternative fluorescent proteins available, such as CFP (Cyan) and YFP (yellow). From an assay perspective, the alternative selected depends on the application. For gene expression studies, DLR® (Dual luciferase reporter assay) or GeneBLAzer® assays may be suitable. For FRET / BRET studies, fluorescent labels might be an alternative.

Instrument parameters




Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**GFP**	**GFP**	**GFP**	**GFP**
Mode	Bottom	Bottom	Bottom	Bottom
Excitation wavelength	483 (9) nm	485 (20) nm	485 (20) nm	485 (20) nm
Emission wavelength	535 (20) nm	535 (25) nm	535 (25) nm	535 (20) nm
Lag time	0 µs	0 µs	0 µs	0 µs
Integration time	20 µs	20 µs	20 µs	20 µs
Flashes	25	25	10	10
Mirror	/	automatic	automatic	/
Gain	optimal	optimal	optimal	optimal
Z-position	-	-	-	-
Settle time	0 ms	0 ms	0 ms	0 ms

Support

Link

- http://www.olympusfluoview.com/java/excitationefficiency/index.html

Tecan Application Notes

- Analyzing biological drug effects in 3D
- Analyzing biological processes
- Improved Detection of Green Fluorescent Protein (GFP) in the Infinite® 200 PRO
- Detection of Green Fluorescent Protein (eGFP)

TRF – Time-Resolved Fluorescence

Light is absorbed and emitted for a relatively long period of time

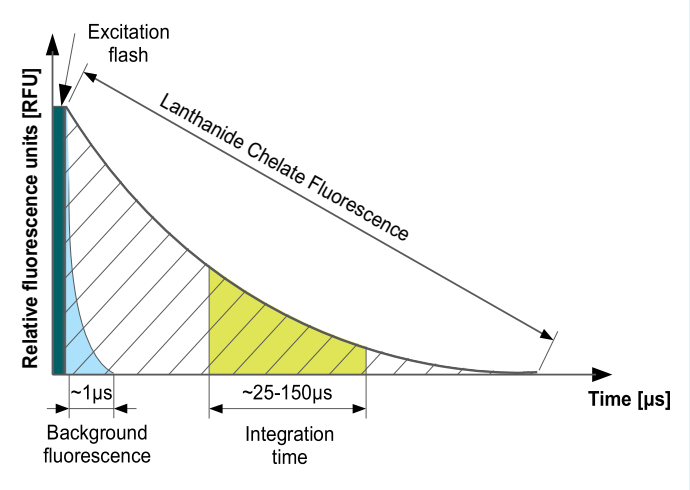


Figure 1: Schematic drawing of a time resolved emission spectrum

Technology

TRF is similar to standard fluorescence, except that the light is emitted for a much longer period of time (Figure 1). The advantage of this is that the signal can be measured once all the background fluorescence (noise) has subsided, increasing the signal to noise ratio, and hence the sensitivity. Only lanthanides – also called rare earth metals – are capable of this kind of fluorescence[25].

From all existing lanthanides Europium (Eu), Samarium (Sm), Terbium (Tb) and Dysprosium (Dy) are the most used ones in life science.

In most cases, it is possible to substitute fluorescence applications with TRF to achieve higher sensitivity and / or lower background noise.

Major applications

- DELFIA® – Dissociation-Enhanced Lanthanide Fluorescent Immunoassay

[25] Lakowicz, J. R. (1999). Principles of Fluorescence Spectroscopy. Kluwer Academic / Plenum Publishers

Compatible readers




Features	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Reader design	Monochromator	Filter	Filter	Monochromator
Configuration	Quad4 Monochromators™, 2 excitation and 2 emission monochromators for high performance, high flexibility and accurate data acquisition	Up to 4 programmable filter pairs per slide that are easily ejected and exchanged through the front of the instrument	Up to 6 filter pairs per slide which are individually moveable, allowing any combination of Ex and Em filters; easy filter change, ID chip	Premium Quad4 Monochromators, 2 excitation and 2 emission monochromators allow for stray light reduction up to a factor of 10^7
Capability	Yes	No	No	Yes
Capability – TR-FRET	No	No	No	Yes
Dichroic mirrors	-	Dichroic mirror, optimized for TRF measurements (Eu, Tb, Sm)	Multiple dichroic mirrors	-
Z-adjustment	Automatic	-	Automatic	Automatic
Plate format	6- to 384-well plates NanoQuant Plate	6- to 384-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate
Temperature control	Ambient +5 °C to 42 °C	Ambient +5 °C to 42 °C	Ambient +4 °C to 42 °C	Ambient +4 °C to 42 °C
Shaking	Linear, orbital with variable amplitudes	Linear, orbital with variable amplitudes	Linear, orbital with variable amplitudes	Linear, orbital, double orbital with variable amplitudes
Injectors	2	2	2	2
Stacker	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	On-board stacker: stacks for 30 or 50 plates
Barcode reader	Yes	Yes	Yes	Yes

Typcal values: Time-Resolved fluorescence (TRF)	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Light source	UV xenon flash lamp	UV xenon flash lamp	High energy xenon flash lamp	High energy xenon flash lamp
Detector	PMT, optionally UV and red-sensitive	PMT, optionally UV and red-sensitive	Extended wavelength (UV and far-red) Low dark current PMT	Extended wavelength (UV and far-red) Low dark current PMT
Wavelength range	Standard: Ex: 230 - 600 nm Em: 330 - 600 nm Optional: Ex: 230 - 850 nm Em: 280 - 850 nm	Standard: Ex: 230 - 600 nm Em: 330 - 600 nm Optional: Ex: 230 - 850 nm Em: 280 - 850 nm	Ex: 230 - 900 nm Em: 280 - 900 nm	Ex: 230 - 850 nm Em: 280 - 850 nm
Sensitivity	90 amol / well (0.9 pM; 384-well plate) europium	2.8 amol / well (28fM; 384-well plate) europium	0.5 amol / well europium (Greiner 384-well low volume white plate; 10 µl), 50fM europium	1.5 amol / well europium (Greiner 384-well low volume white plate; 10 µl), 150fM europium
Bandwidth	Ex: <5 nm for λ ≤315 nm and <9 nm for λ >315 Em: <10 nm for λ ≤315 nm and <20 nm for λ >315	Filter dependent	Filter dependent	Adjustable ≤300 nm: 2.5 - 10 nm; >300 nm: 5 - 20 nm
Wavelength selection	Monochromator	Filter	Filter	Monochromator

Support

Tecan Application Note • TRF Sensitivity

DELFIA® – Dissociation-Enhanced Lanthanide Fluorescent Immunoassay[26]

TRF-based alternative to absorbance-based ELISA

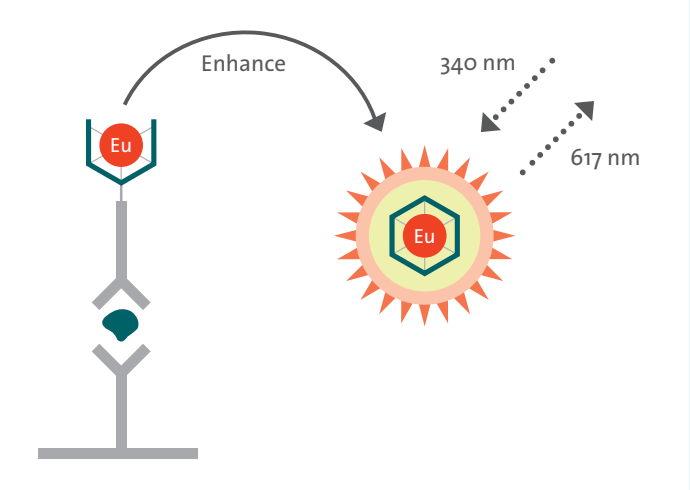


Figure 1: Schematic principle of DELFIA

Assay overview

Technology
TRF – Time-Resolved Fluorescence

Principle, Provider
PerkinElmer offers the most common lanthanide chelates, including Europium (Eu), Samarium (Sm), Terbium (Tb) and Dysprosium (Dy), under the brand name DELFIA, making DELFIA a technology rather than a single assay.

Major applications
In addition to the self-labeling kits, which allow users to label almost any biomolecule with the lanthanide chelates, PerkinElmer offers pre-coupled antibodies and DNA probes. DELFIA is also available as a ready-to-go assay for cytotoxicity or cell proliferation studies. Other major applications include: receptor-ligand binding, enzyme assays, protein-protein and protein-DNA interaction studies.

Alternatives
As a common application, it is used as an alternative approach to the well-established, absorbance- or fluorescence-based ELISA[27].

Mechanism
The biomolecule (antibody, DNA probe, etc.) used for detection is labeled with one of the lanthanide chelates. Assays are performed in an endpoint manner and only need to be read once, when all pipetting steps are complete. All steps are performed according to a standard ELISA protocol. Instead of a substrate an enhancement solution is added, that disconnects the chelate lanthanide-chelate complex from the antibody to increase the signal intensity.

[26] http://www.perkinelmer.com/Catalog/Category/ID/delfia%20trf%20assays%20and%20reagents
[27] Enzyme linked Immunosorbent assay

Instrument parameters




Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**DELFIA**	**DELFIA**	**DELFIA**	**DELFIA**
Mode	FI Top	FI Top	FI Top	FI Top
Excitation wavelength	Eu: 340(9) nm	Eu: 340(35)nm	Eu: 340(35)nm	Eu: 345(20)nm
Emission wavelength	Eu: 617(20) nm	Eu: 612(10) nm	Eu: 612(10) nm	Eu: 617(20) nm
Lag time	200 µs	200 µs	200 µs	200 µs
Integration time	400 µs	400 µs	400 µs	400 µs
Flashes	25	25	10	10 (100 Hz)
Mirror	/	automatic	automatic	/
Gain	automatic	automatic	automatic	automatic
Z-position	automatic	/	automatic	automatic
Settle time	0 ms	0 ms	0 ms	0 ms

Support

Links

- DELFIA web page: http://www.perkinelmer.com/Catalog/Category/ID/delfia%20trf%20assays%20and%20reagents
- DELFIA knowledge base: http://perkinelmerreagents.onconfluence.com/pages/viewpage.action?pageId=328586

Tecan Application Note

- TRF Sensitivity

FRET – Fluorescence Resonance Energy Transfer

Light is absorbed, transferred to another fluorophore and then emitted

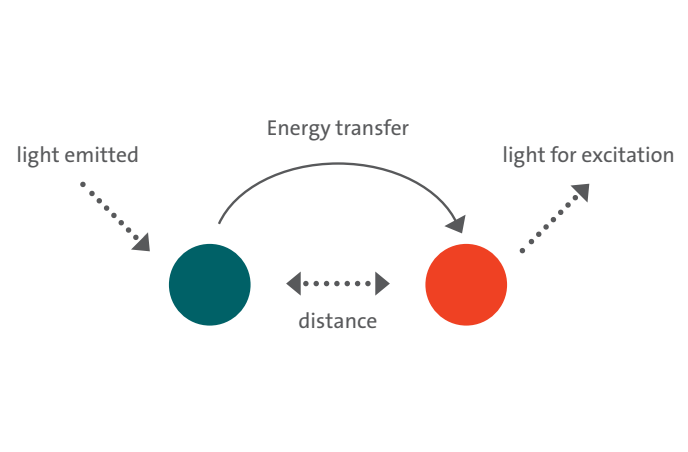


Figure 1: Schematic principle of FRET. Light emitted from the green molecule is used to excite the red molecule.

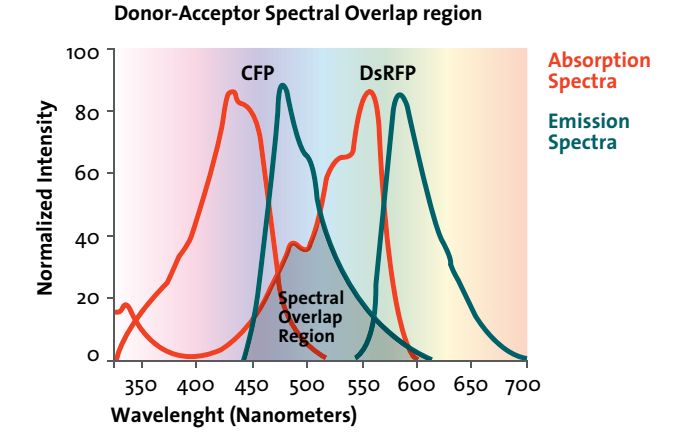


Figure 2: FRET is possible because the emission spectrum of CFP and the excitation spectrum of DsRFP overlap between 450 and 600 nm.

Technology

As the name implies, FRET involves energy transfer between two fluorescent molecules (Figure 1). However, there are some specific requirements for this transfer to take place. Firstly, the emission spectrum of the donor fluorophore and the excitation spectrum of the acceptor fluorophore need to overlap (Figure 2), as the emission light of the donor fluorophore is used to excite the acceptor fluorophore. Secondly, the distance between the two fluorophores – the Förster radius – should be less than 10 nm (Figure 3)[28].

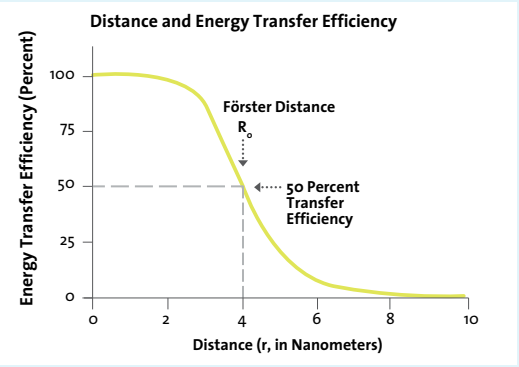


Figure 3: Förster radius – the distance where the FRET signal intensity is reduced to 50 %.

One way in which FRET is used is to determine if two biomolecules are in close proximity. In this case, both biomolecules must be labeled with fluorophores and then combined. After an incubation period, the assay is performed. Samples are excited at the donor excitation wavelength, and measured at the donor and acceptor emission wavelength. To compensate for well-to-well variation, for example from pipetting errors, the ratio of both values is calculated (ratiometric assay). If donor and acceptor are in close proximity, FRET will take place, otherwise only the emission signal of the donor is measurable.

Major applications

- GeneBLAzer and Tango™ GPCR Assay System

Support

Tecan Application Notes

- GeneBLAzer® Assay on Tecan's Infinite® F200 for cell-based screening – A FRET-technology for gene reporter assays
- Inducing hypoxia inside Tecan's Infinite® 200 PRO multimode reader

[28] Lakowicz, J. R. (1999). Principles of Fluorescence Spectroscopy. Kluwer Academic / Plenum Publishers

Compatible readers




Features	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Reader design	Monochromator	Filter	Filter	Monochromator
Configuration	Quad4 Monochromators™, 2 excitation and 2 emission monochromators for high performance, high flexibility and accurate data acquisition	Up to 4 programmable filter pairs per slide that are easily ejected and exchanged through the front of the instrument	Up to 6 filter pairs per slide which are individually moveable, allowing any combination of Ex and Em filters; easy filter change, ID chip	Premium Quad4 Monochromators, 2 excitation and 2 emission monochromators allow for stray light reduction up to a factor of 10^7
Capability – scanning	Yes	No	No	Yes
Capability – scanning 3D	No	No	No	Yes
Dichroic mirrors	-	Dichroic mirror, optimized for TRF measurements (Eu, Tb, Sm)	Multiple dichroic mirrors	-
Z-adjustment	Automatic	-	Automatic	Automatic
Plate format	6- to 384-well plates NanoQuant Plate	6- to 384-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate
Temperature control	Ambient +5 °C to 42 °C	Ambient +5 °C to 42 °C	Ambient +4 °C to 42 °C	Ambient +4 °C to 42 °C
Shaking	Linear, orbital with variable amplitudes	Linear, orbital with variable amplitudes	Linear, orbital with variable amplitudes	Linear, orbital, double orbital with variable amplitudes
Injectors	2	2	2	2
Stacker	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	On-board stacker: stacks for 30 or 50 plates
Barcode reader	Yes	Yes	Yes	Yes

Typcal values: Fluorescence intensity (FI)	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Capability top	Yes	Yes	Yes	Yes
Capability bottom	Yes	Yes	Yes	Yes
Light source	UV xenon flash lamp	UV xenon flash lamp	High energy xenon flash lamp	High energy xenon flash lamp
Detector	PMT, optionally UV and red-sensitive	PMT, optionally UV and red-sensitive	Extended wavelength (UV and far-red) Low dark current PMT	Extended wavelength (UV and far-red) Low dark current PMT
Wavelength range	Standard: Ex: 230 - 600 nm Em: 330 - 600 nm Optional: Ex: 230 - 850 nm Em: 280 - 850 nm	Standard: Ex: 230 - 600 nm Em: 330 - 600 nm Optional: Ex: 230 - 850 nm Em: 280 - 850 nm	Ex: 230 - 900 nm Em: 280 - 900 nm	Ex: 230 - 850 nm Em: 280 - 850 nm
Sensitivity – top	170 amol / well (1.7pM; 384-well plate)	85 amol / well (0.85pM, 384-well plate)	10 amol / well fluorescein (Greiner® 384-well low volume black plate; 10 µl), 1pM	25 amol / well fluorescein (Greiner 384-well low volume black plate; 10 µl), 2.5pM
Sensitivity – bottom	1.2 fmol / well (6pM; 96-well plate)	0.7 fmol / well (3.5pM; 96-well plate)	0.4 fmol / well fluorescein (Greiner 96-well SensoPlate™; 200 µl), 2pM	0.6 fmol / well fluorescein (Greiner 96-well SensoPlate; 200 µl), 3pM
Wavelength accuracy	< ±2 nm for λ >315 nm; < ±1 nm for λ ≤315 nm	Filter dependent	Filter dependent	≤300 nm: ±0.5 nm; >300 nm: ±1 nm
Wavelength reproducibility	< ±1 nm for λ >315 nm; < ±0.5 nm for λ ≤315 nm	Filter dependent	Filter dependent	≤300 nm: ±0.5 nm; >300 nm: ±1 nm
Bandwidth	Ex: <5 nm for λ ≤315 nm and <9 nm for λ >315 Em: <10 nm for λ ≤315 nm and <20 nm for λ >315	Filter dependent	Filter dependent	Adjustable ≤300 nm: 2.5 - 10 nm; >300 nm: 5 - 20 nm
Wavelength selection	Monochromator	Filter	Filter	Monochromator

GeneBLAzer® and Tango™ GPCR[29] Assay System[30]

Gene activator assay with FRET readout

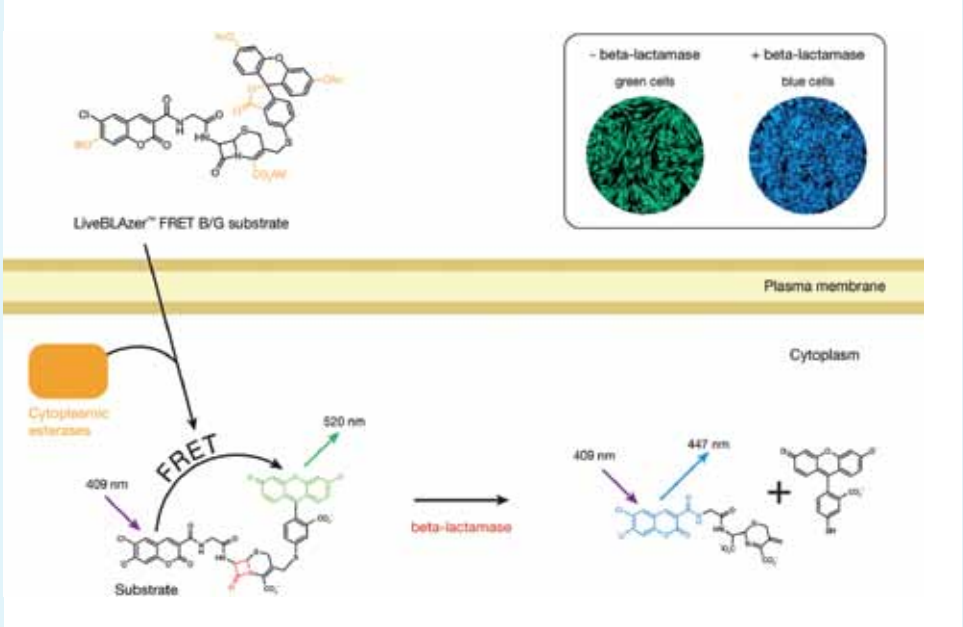


Figure 1: Principle of the GeneBLAzer assay. If the substrate is cleaved, the FRET signal is disrupted.

Assay overview

Technology
Fluorescence Resonance Energy Transfer (FRET), ratiometric

Major application, Principle
Life Technologies' GeneBLAzer assays are designed to monitor the activation of genes, including surface and intracellular reporters, a wide range of signal transduction pathways, ion channels and other transporters. The basis for the GeneBLAzer assay are cell lines possessing a β-lactamase[31] (BLA) gene under the control of a promotor which is downstream of the monitored target protein.

Provider
The β-lactamase-transfected cell lines can either be purchased from Life Technologies or self-transfected. Tango cell lines are also based on the GeneBLAzer technology, but are designed exclusively for GPCR activation assays.

Mechanism
As shown in Figure 1, after the transfection, cells are loaded with an engineered fluorescent substrate which is an assembly of two fluorophores: coumarin and fluorescein. If the target gen is inactive, BLA is not expressed and the substrate molecule is not cleaved. In this state, excitation of the coumarin results in FRET to the fluorescein moiety and emission of green light.
However, in the presence of BLA expression, the substrate is cleaved, causing the separation of the fluorophores, and FRET cannot occur. This results in the emission of a blue fluorescence signal from coumarin.
Assays are commonly measured over several hours, or even days. During this time period the plate can either be shuttled between the incubator and the reader, or a temperature and gas controlled multimode reader such as the Infinite 200 PRO may be used.

Alternatives
Various fluorescence (e.g. GFP) or luminescence (e.g. DLR™) reporter gen assays.

[29] G-Protein Coupled Receptors: important group of cell surface receptors for cellural signalling
[30] http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Drug-Discovery/Target-and-Lead-Identification-and-Validation/g-protein_coupled_html/GPCR-Cell-Based-Assays/GeneBLAzer-Theory.html
[31] β-lactamase is an enzyme that can cleave specific substrates

Instrument parameters




Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**GeneBLAzer**	**GeneBLAzer**	**GeneBLAzer**	**GeneBLAzer**
Measurement	Donor	Donor	Donor	Donor
Mode	FI bottom	FI bottom	FI bottom	FI bottom
Excitation wavelength	409(9) nm	415(20) nm	415(20) nm	409(12) nm
Emission wavelength	460(20) nm	460(20) nm	460(20) nm	460(12) nm
Lag time	0 µs	0 µs	0 µs	0 µs
Integration time	40 µs	40 µs	40 µs	40 µs
Flashes	10	25	10	10
Mirror	automatic	automatic	automatic	automatic
Gain	optimal	optimal	optimal	optimal
Z-position	calculated	/	calculated	calculated
Settle time	0 ms	0 ms	0 ms	0 ms
Assay	**GeneBLAzer**	**GeneBLAzer**	**GeneBLAzer**	**GeneBLAzer**
Measurement	Acceptor	Acceptor	Acceptor	Acceptor
Mode	FI bottom	FI bottom	FI bottom	FI bottom
Excitation wavelength	409(9) nm	415(20) nm	415(20) nm	409(12) nm
Emission wavelength	530(20) nm	535(25) nm	535(25) nm	530(12) nm
Lag time	0 µs	0 µs	0 µs	0 µs
Integration time	40 µs	40 µs	40 µs	40 µs
Flashes	10 (100 Hz)	25	10	10 (100 Hz)
Mirror	automatic	automatic	automatic	automatic
Gain	optimal	optimal	optimal	optimal
Z-position	calculated	/	calculated	calculated
Settle time	0 ms	0 ms	0 ms	0 ms

Support

Links

- GeneBLAzer homepage: http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Drug-Discovery/Target-and-Lead-Identification-and-Validation/g-protein_coupled_html/GPCR-Cell-Based-Assays/GeneBLAzer-Theory.html?CID=flgeneblazer
- Video - TANGO cell line: http://media.invitrogen.com.edgesuite.net/stand_alone/Tango-GPCR-Cell-Lines.html
- LiveBLAzer manual: http://tools.invitrogen.com/content/sfs/manuals/liveblazer_FRETBGLoadingKit_man.pdf

Tecan Application Notes

- GeneBLAzer® Assay on Tecan's Infinite® F200 for cell-based screening – A FRET-technology for gene reporter assays
- Inducing hypoxia inside Tecan's Infinite® 200 PRO multimode reader

TR-FRET – Time-Resolved Fluorescence Resonance Energy Transfer

FRET with a longer lifetime and hence a lower background

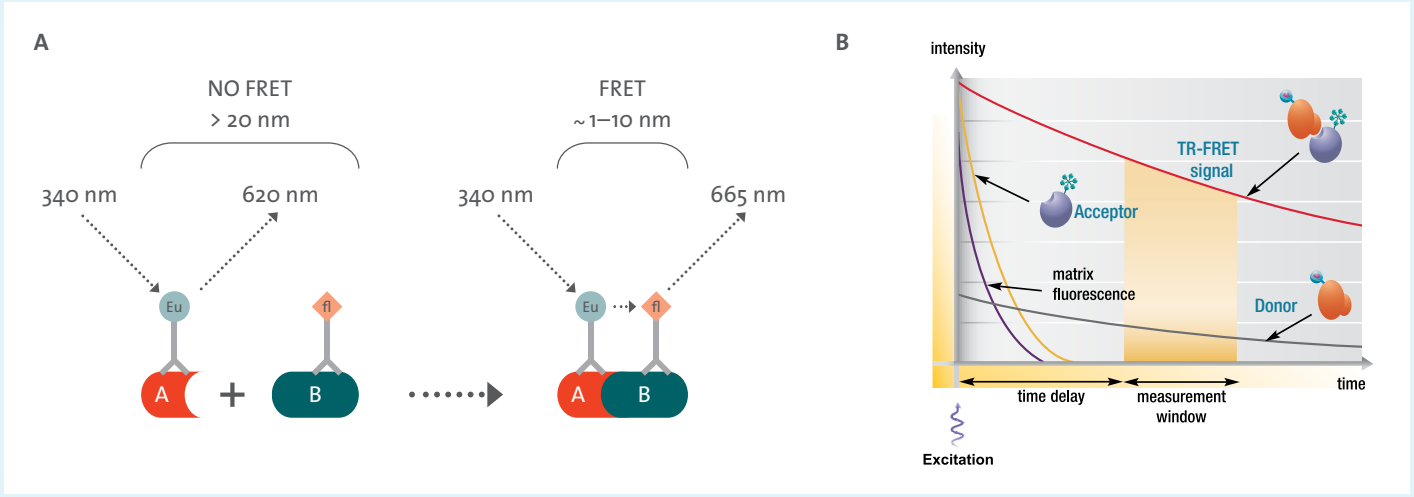


Figure 1: From a technology perspective TR-FRET is equivalent to FRET. In order to reduce the background of the assay, a lanthanide lable (e.g. Eu) and a standard fluorescence molecule (fl) as acceptor molecule.

Figure 2: Signal vs. time plot showing the advantages of using lanthanide lables in a FRET processmolecule.

Technology

TR-FRET combines the advantages of Time-Resolved Fluorescence (TRF) with the functionality of Fluorescence Resonance Energy Transfer (FRET) (Figure 1). While most standard FRET assays have difficulties with high background, this issue can be resolved by using time-resolved donor molecules. As shown in Figure 2, by the time donor and acceptor emission signals are measured all the unspecific background noise has vanished, giving a high signal to background noise ratio and therefore greater sensitivity.

Assay design

TR-FRET assays are commonly designed to detect whether molecules are in close proximity. This can be exploited to determine if, for example, a protein, peptide substrate, small molecule, phosphorylation, or acetylation is present, or if binding has occurred, for example a receptor-ligand interaction. A major limitation of this technology is that the maximum distance between the donor and the acceptor molecule cannot exceed 10 nm[32].

Major applications

- HTRF® – Homogeneous Time-Resolved Fluorescence
- Adapta® Universal Kinase Assay and Substrates
- LanthaScreen™ Kinase Activity Assays

[32] Lakowicz, J. R. (1999). Principles of Fluorescence Spectroscopy. Kluwer Academic / Plenum Publishers

Compatible readers

Features	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Reader design	Filter	Filter	Monochromator
Configuration	Up to 4 programmable filter pairs per slide that are easily ejected and exchanged through the front of the instrument	Up to 6 filter pairs per slide which are individually moveable, allowing any combination of Ex and Em filters; easy filter change, ID chip	Premium Quad4 Monochromators, 2 excitation and 2 emission monochromators allow for stray light reduction up to a factor of 10^7
HTRF	Yes	Yes	Yes
LanthaScreen	Yes	Yes	Yes
Transcreener TR-FRET	No	Yes	Yes
Capability	No	No	Yes
Capability – TR-FRET	No	No	Yes
Dichroic mirrors	Dichroic mirror, optimized for TRF measurements (Eu, Tb, Sm)	Multiple dichroic mirrors	-
Z-adjustment	-	Automatic	Automatic
Plate format	6- to 384-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate
Temperature control	Ambient +5 °C to 42 °C	Ambient +4 °C to 42 °C	Ambient +4 °C to 42 °C
Shaking	Linear, orbital with variable amplitudes	Linear, orbital with variable amplitudes	Linear, orbital, double orbital with variable amplitudes
Injectors	2	2	2
Stacker	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	On-board stacker: stacks for 30 or 50 plates
Barcode reader	Yes	Yes	Yes

Typcal values: Time-Resolved fluorescence (TRF)	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Light source	UV xenon flash lamp	High energy xenon flash lamp	High energy xenon flash lamp
Detector	PMT, optionally UV and red-sensitive	Extended wavelength (UV and far-red) Low dark current PMT	Extended wavelength (UV and far-red) Low dark current PMT
Wavelength range	Standard: Ex: 230 - 600 nm Em: 330 - 600 nm Optional: Ex: 230 - 850 nm Em: 280 - 850 nm	Ex: 230 - 900 nm Em: 280 - 900 nm	Ex: 230 - 850 nm Em: 280 - 850 nm
Sensitivity	2.8 amol / well (28fM; 384-well plate) europium	0.5 amol / well europium (Greiner 384-well low volume white plate; 10 µl), 50fM europium	1.5 amol / well europium (Greiner 384-well low volume white plate; 10 µl), 150fM europium
Bandwidth	Filter dependent	Filter dependent	Adjustable ≤300 nm: 2.5 - 10 nm; >300 nm: 5 - 20 nm
Wavelength selection	Filter	Filter	Monochromator

Support

Tecan Application Notes

- Implementation of Tag-lite™ Technology (Cisbio bioassays) on Tecan's Infinite® F500 Multimode Reader
- GeneBLAzer® Assay on Tecan's Infinite® F200 for cell-based screening – A FRET-technology for gene reporter assays
- LanthaScreen® TR-FRET Assay
- Development of a functional assay (HTRF®, Cisbio) to detect cAMP concentration after activation of 5-HT1A receptors
- HTRF® (Cisbio) Human Interleukin beta (IL1β) assay
- HTRF® Homogenous TR-FRET Assay
- Homogeneous time-resolved fluorescence (HTRF®) in Tecan's Infinite® F500 filter-based multimode reader (White Paper)

HTRF® – Homogeneous Time-Resolved Fluorescence[33]

TR-FRET-based assay platform

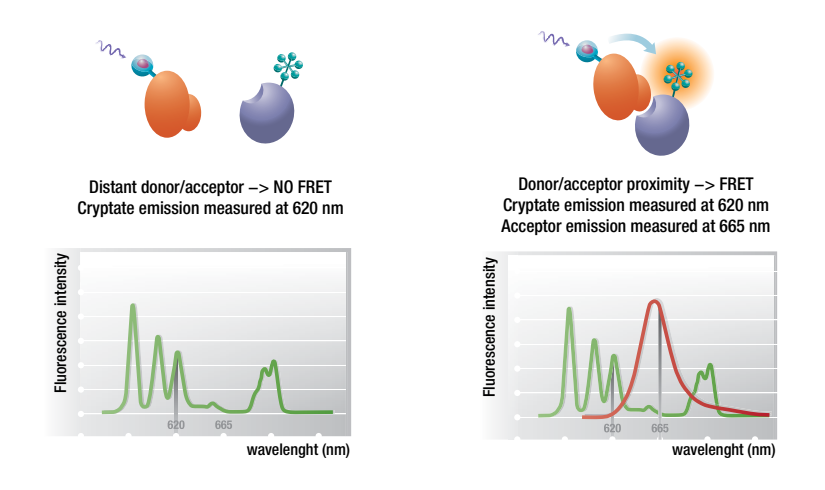


Figure 1: Mechanistic principle of the HTRF technology and fluorescence spectrum

Assay overview

Technology
TR-FRET – Time-Resolved Fluorescence Resonance Energy Transfer, ratiometric

Provider
HTRF is Cisbio's TR-FRET-based assay platform, which provides a broad range of solutions.

Format, major applications
Biomolecules for detection can either be self-labeled or purchased pre-labeled. Additionally, ready-to-go assays and pre-coupled antibodies are available for major targets, including GPCRs, with second messengers and binding assays, kinases, epigenetic enzymes, protein-protein interactions and biomarkers.

Mechanism
HTRF is based on Eu^{3+} / Tb^{2+} cryptate donors and XL665 or d2 acceptors, which can be coupled to almost any biomolecule desired, including proteins, peptides, DNA and small molecules. The technology is based on no wash assay procedure detecting proximity events between donor and acceptor dyes.
The assay detection is obtained upon dispensing acceptor and donor conjugates to the sample to be assessed (e.g. enzymatic reaction mixture, cell lysate, or supernatant). No washing steps are required (homogeneous assay), and detection is performed after the completion of incubation, by measuring both specific donor and acceptor fluororescences (Figure 1). To compensate for well-to-well variation, the ratio of both values is calculated (hence ratiometric assay). Donor fluorescence will always be detected and used as an internal control, while an emission signal from the acceptor is only detected if both biomolecules are in close proximity and FRET occurs[33].

[33] http://www.htrf.com

Instrument parameters



Reader	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**HTRF**	**HTRF**	**HTRF**
Measurement	Donor	Donor	Donor
Mode	FI top	FI top	FI top
Excitation wavelength	Eu: 320(25) nm Tb: 340(35) nm	Eu: 320(25) nm Tb: 340(35) nm	Eu: 317(20) nm Tb: 332(20) nm
Emission wavelength	620(10) nm	620(10) nm	620(10) nm
Lag time	150 µs	150 µs	60 µs
Integration time	500 µs	500 µs	500 µs
Flashes	50	10	125-165 (100 Hz)
Mirror	automatic	automatic	/
Gain	optimal	optimal	optimal
Z-position	-	calculated	calculated
Settle time	0 ms	0 ms	0 ms
Assay	**HTRF**	**HTRF**	**HTRF**
Measurement	Acceptor	Acceptor	Acceptor
Mode	FI top	FI top	FI top
Excitation wavelength	Eu: 320(25) nm Tb: 340(35) nm	Eu: 320(25) nm Tb: 340(35) nm	Eu: 317(20) nm Tb: 332(20) nm
Emission wavelength	665(8) nm	665(8) nm	665(10) nm
Lag time	150 µs	150 µs	60 µs
Integration time	500 µs	500 µs	500 µs
Flashes	25	10	10 (100 Hz)
Mirror	automatic	automatic	/
Gain	optimal	optimal	optimal
Z-position	/	calculated	calculated
Settle time	0 ms	0 ms	0 ms

Support

Link

- Assay homepage: http://www.htrf.com/htrf-homepage

Tecan Application Notes

- Implementation of HTRF® Assay Technology on Infinite® F200 PRO
- Development of a functional assay (HTRF®, Cisbio) to detect cAMP concentration after activation of 5-HT1A receptors
- HTRF® (Cisbio) Human Interleukin beta (IL1ß) assay
- Homogeneous time-resolved fluorescence (HTRF®) in Tecan's Infinite® F500 filter-based multimode reader (White Paper)
- Implementation of Tag-lite® Technology (Cisbio bioassays) on Tecan's Infinite® F500 Multimode Reader
- Implementation of HTRF® on Tecan's Infinite® M1000 premium quad4 monochromators™ multimode microplate reader
- Impact of Extended Adjustable Monochromator Bandwidth in Fluorescence Based Application Technologies
- HTRF® Homogenous TR-FRET Assay

Adapta® Universal Kinase Assay and Substrates

Life Technologies' version of the ADP detection assay

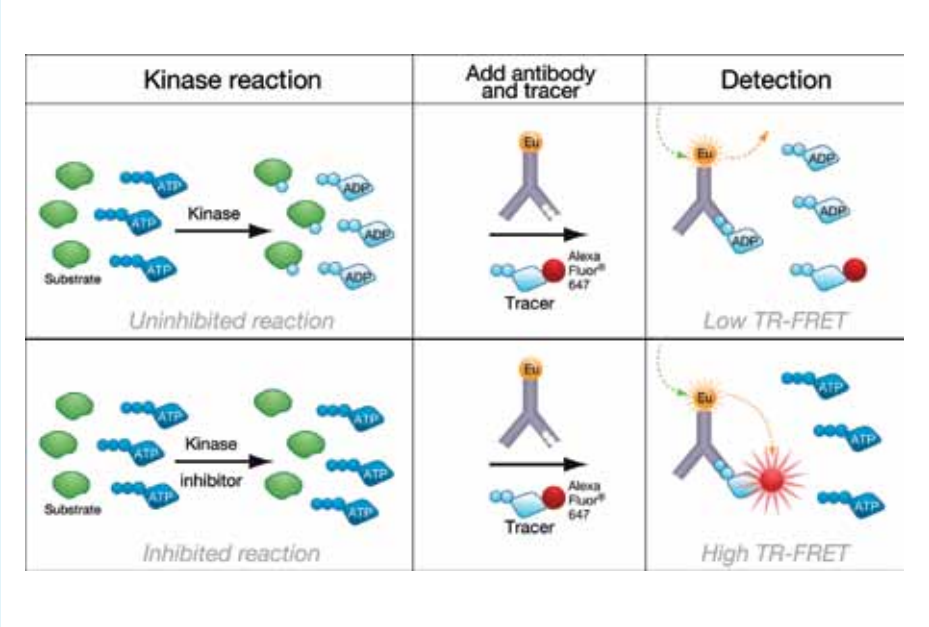


Figure 1: Schematic principle of the adapta assay

Assay overview

Technology
Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET), ratiometric

Principle, provider, major application
Life Technologies' Adapta Universal Kinase Assay Kit is a homogeneous, fluorescence-based immunoassay for measuring the activity of ADP-producing enzymes, mainly kinases. Additionally, the Adapta assay is available for a selection of lipid- and peptide-based substrates. Life Technologies supplies europium-coupled antibody specific for ADP.

Mechanism
The ADP-specific antibody and the tracer are added to the sample. In an inhibited reaction (Figure 1), the monitored kinase produces no ADP and only the added, tracer-bound ADP molecule binds to the antibody, causing a high FRET signal.
Active kinases convert ATP to ADP. The free ADP competes with the tracer-bound ADP to bind to the antibody, resulting in a low FRET signal. Hence, the signal intensity is indirectly proportional to the activity of the kinase.

Instrument parameters



Reader	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**Adapta**	**Adapta**	**Adapta**
Measurement	Donor	Donor	Donor
Mode	FI top	FI top	FI top
Excitation wavelength	340(35) nm	340(35) nm	317(20) nm
Emission wavelength	620(10) nm	620(10) nm	620(12) nm
Lag time	100 µs	100 µs	100 µs
Integration time	200 µs	200 µs	200 µs
Flashes	10	10	20 (100 Hz)
Mirror	automatic	automatic	/
Gain	optimal	optimal	optimal
Z-position	calculated	calculated	calculated
Settle time	0 ms	0 ms	0 ms
Assay	**Adapta**	**Adapta**	**Adapta**
Measurement	Acceptor	Acceptor	Acceptor
Mode	FI top	FI top	FI top
Excitation wavelength	340(35) nm	340(35) nm	317(20) nm
Emission wavelength	665(8) nm	665(8) nm	665(12) nm
Lag time	100 µs	100 µs	100 µs
Integration time	200 µs	200 µs	200 µs
Flashes	10	10	20 (100 Hz)
Mirror	automatic	automatic	/
Gain	optimal	optimal	optimal
Z-position	calculated	calculated	0 ms
Settle time	0 ms	0 ms	0 ms

Support

Link

- Assay link: www.invitrogen.com/adapta

LanthaScreen™ Kinase Activity Assays[34]

Kinase activity assay with TR-FRET readout

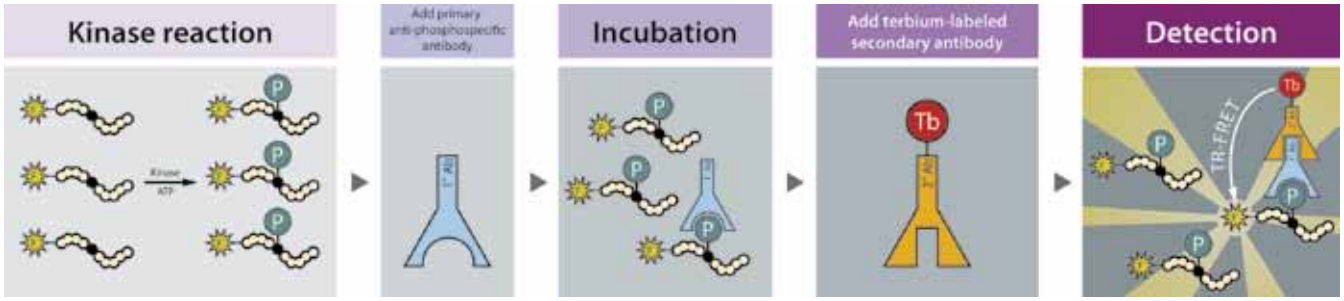


Figure 1: Schematic principle of the LanthaScreen assay

Assay overview

Technology
Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET), ratiometric

Principle, Provider, Major applications
LanthaScreen is a kinase activity assay sold by Life Technologies. Kinases are important cellular enzymes, and their major function is to add phosphate groups to peptide substrates. For researchers, it is important to know how active kinases are in the presence of certain inhibitors. LanthaScreen quantifies kinase activity by measuring the amount of phosphorylated substrate.

Format
Life Technologies supplies a broad panel of fluorescein-labeled substrates and the corresponding lanthanide-labeled antibody specifically for the detection of phosphorylated substrates.

Mechanism
Kinase and fluorescein-labeled substrates are incubated to enable phosphorylation. After incubation, a terbium-labeled antibody is added to the reaction (Figure 1).

Scenario 1 – kinase is active
The substrate was phosphorylated, allowing the phospho-specific antibody to bind. The fluorescein and terbium labels are now in close proximity, resulting in a high FRET signal.

Scenario 2 – kinase is inactive
No phosphorylation occurred, and therefore the antibody could not bind to the substrate. FRET cannot occur. The final result is a dimensionless number that is calculated as the ratio of the acceptor (fluorescein) signal to the donor (terbium) signal.

[34] www.invitrogen.com/lanthascreen

Instrument parameters

Reader	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**LanthaScreen**	**LanthaScreen**	**LanthaScreen**
Measurement	Donor	Donor	Donor
Mode	FI top	FI top	FI top
Excitation wavelength	340(35) nm	340(35) nm	332(20) nm
Emission wavelength	495(10) nm	495(10) nm	485(20) nm
Lag time	100 µs	100 µs	100 µs
Integration time	200 µs	200 µs	200 µs
Flashes	25	10	50 (100 Hz)
Mirror	automatic	automatic	/
Gain	optimal	optimal	optimal
Z-position	-	calculated	calculated
Settle time	0 ms	0 ms	0 ms
Assay	**LanthaScreen**	**LanthaScreen**	**LanthaScreen**
Measurement	Acceptor	Acceptor	Acceptor
Mode	FI top	FI top	FI top
Excitation wavelength	340(35) nm	340(35) nm	332(20) nm
Emission wavelength	520(10) nm	520(10) nm	515(20) nm
Lag time	100 µs	100 µs	100 µs
Integration time	200 µs	200 µs	200 µs
Flashes	25	10	50 (100 Hz)
Mirror	automatic	automatic	/
Gain	optimal	optimal	optimal
Z-position	-	calculated	calculated
Settle time	0 ms	0 ms	0 ms

Support

Link

- Assay link: www.invitrogen.com/lantha screen

Tecan Application Note

- LanthaScreen® TR-FRET Assay-Implementation on Tecan's Infinite® F200 Multimode Reader
- Implementation of LanthaScreen® Technology on Tecan's Infinite® F200 multimode microplate reader. In cooperation with Invitrogen Corporation
- LanthaScreen® TR-FRET Assay (F500)

FP – Fluorescence Polarization

Binding assay for biomolecules

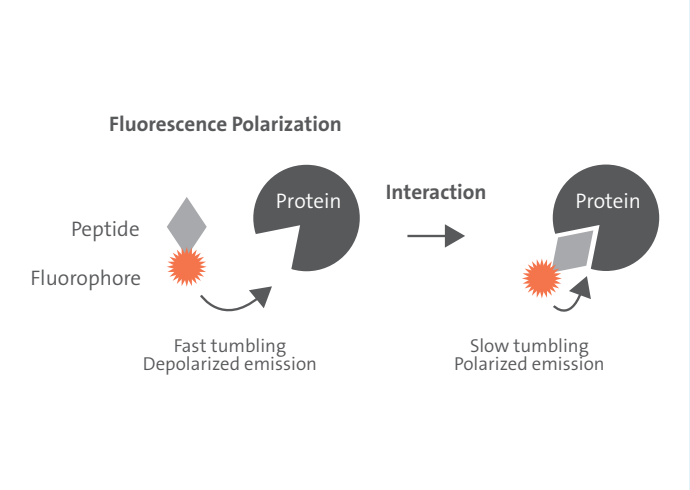


Figure 1: Schematic reprenstation of Fluorescence Polarization

Technology

Fluorescence anisotropy is colloquially referred to as Fluorescence Polarization. When exciting a fluorophore with polarized light, the emitted light will also be polarized in the same direction. Rotational movements of the excited molecule destroy this correlation. The extent of polarization remaining depends on the size of the molecules measured: the bigger the molecules, the slower they rotate and the higher the conservation of the original polarization. Other influences include solvent viscosity, temperature and the lifetime of the excited state.[35]

The following metaphor is an easy way to explain FP. Imagine a little child playing in a field. While it is free, it can twist and turn as much as it wants in any direction. Once it is 'attached' to its mother's hand, the movements will slow down and get direction. This comparison can be used to show how FP detects molecular interactions (Figure 1). The little child represents the smaller of the interaction partners, and the mother the larger one. A fluorescent probe is attached to the small molecule to observe the turning and twisting movements. As long as there is no interaction between the small and the large molecule, the rotation of the fluorophore is fast and the emitted light depolarized. Once it binds to a larger interaction partner, its movements will slow down and the emitted light will conserve the polarization of the excitation light.

Assay design

A major application of FP is the detection of molecular interactions. FP assays require interaction partners to be different sizes, and the smaller molecule to be labeled with a fluorophore. Commercial assays commonly provide these labeled partners or substrates. The final result is a ratio of the polarization values, measured before and after addition of the suspected interaction partner. The polarization is calculated using the equation given below, measuring the intensity of emitted light in perpendicular and parallel planes.

Changes in polarization give information about the creation of interactions and their strength. A higher mP (milli-polarization) value represents a stronger interaction between the two molecules.

Major applications

- PolarScreen™
- Transcreener®

$$mP = 1000 \times \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

Equation 1: Calculation of the polarization value.
$I_{\parallel}$ = light parallel to the polarization plane. $I_{\perp}$ = light perpendicular to the polarization plane

[35] Lakowicz, J. R. (1999). Principles of Fluorescence Spectroscopy. Kluwer Academic / Plenum Publishers

Compatible readers

Features	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Reader design	Filter	Filter	Monochromator
Configuration	Up to 4 programmable filter pairs per slide that are easily ejected and exchanged through the front of the instrument	Up to 6 filter pairs per slide which are individually moveable, allowing any combination of Ex and Em filters; easy filter change, ID chip	Premium Quad4 Monochromators, 2 excitation and 2 emission monochromators allow for stray light reduction up to a factor of 10^7
Transcreener FP	No	Yes	Yes
Z-adjustment	-	Automatic	Automatic
Temperature control	Ambient +5 °C to 42 °C	Ambient +4 °C to 42 °C	Ambient +4 °C to 42 °C
Shaking	Linear, orbital with variable amplitudes	Linear, orbital with variable amplitudes	Linear, orbital, double orbital with variable amplitudes
Injectors	2	2	2

Typcal values: Fluorescence Polarization (FP)	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Capability	Yes	Yes	Yes
Light source	Flash lamp	Flash lamp	4 Different light-emitting diodes (LED) (470, 530, 590, 635 nm)
Detector	PMT, optionally UV and redsensitive	Extended wavelength (UV and far-red) Low dark current PMT	Extended wavelength (UV and far-red) Low dark current PMT
Polarizer	Filter	Filter	Liquid crystal
Wavelength range	Standard: Ex 300 - 600 nm, Em 330 - 600 nm Optional: Em 330 - 850 nm	300 - 750 / 330 - 750	Ex (LED): 470, 530, 590, 635 nm Em (mono): 280 - 850 nm
Sensitivity	<4 mP standard deviation @ 1nM fluorescein	<2 mP standard deviation @ 1nM fluorescin	< 2 mP standard deviation @ 1nM fluorescein
Wavelength selection			Excitation: LED; Emission: Monochromator

Support

Tecan Application Notes

- Transcreener® ADP2 Fluorescence Polarization assay
- PolarScreen™ glucocorticoid receptor competitor assay, green/red
- Homogeneous ADP Detection Using Transcreener® Technology on Tecan's Infinite® F500 and Infinite M1000 Multimode Microplate Readers
- The Predictor™ hERG Fluorescence Polarization Assay
- PolarScreen™ Far Red Tyrosine Kinase Assay
- Screening of Vitamin D Receptor ligands with PolarScreen Red™ (Invitrogen)
- PolarScreen Red™ (Invitrogen) Glucocorticoid Receptor Assay

PolarScreen™

FP-based kinase activity assay; FP-equivalent to LanthaScreen

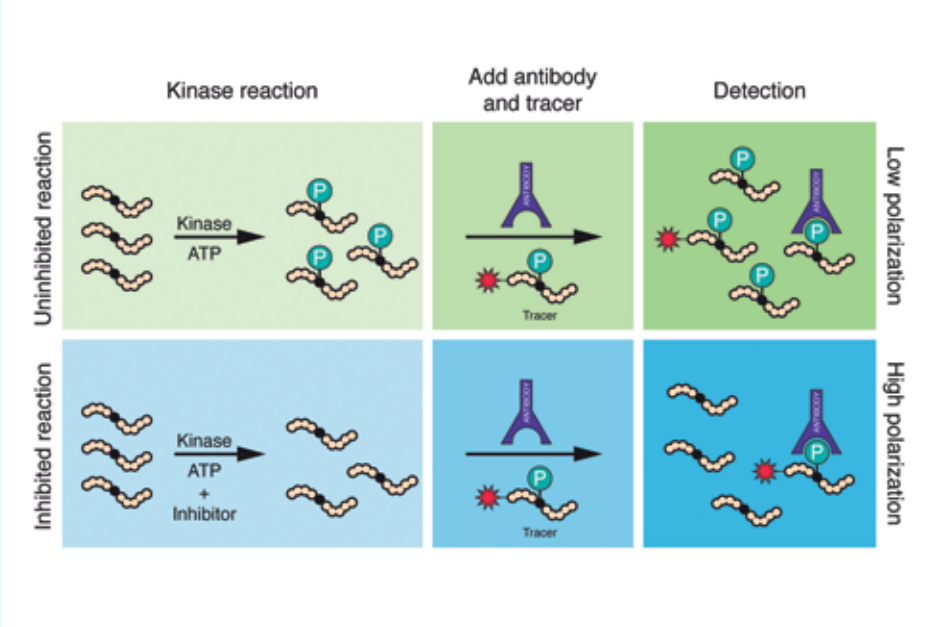


Figure 1: Schematic principle of the PolarScreen assay.

Assay overview

Technology
Fluorescence Polarization (FP)

Provider, Major application
PolarScreen[36] is Life Technologies' version of a FP-based kinase activity assay. Life Technologies offers a panel of phospho-specific antibodies which detect peptide substrates when phosphorylated by protein kinases.

Mechanism
The target kinase is incubated with a dedicated, unlabeled substrate (Figure 1). Antibody – specific for the phosphorylated-phosphorylation site of the substrate – and additionally, tracer-bound substrate are added to the sample. If the unlabeled substrate's phosphorylation site remains unphosphorylated, for example due to an inactive enzyme, the antibody will only bind to the added, tracer-bound substrate, causing a low FP signal.

Active kinases will phosphorylate the non-tracer bound substrate, which then competes with the tracer-bound substrate for binding to the antibody, resulting in a high FP signal. Hence, the FP signal is inversely proportional to the amount of phosphorylated substrate.

Format
Fluorescence labels are available for green (fluorescein), red (rhodamine derivative) or far-red detection. Red fluorescence readouts help to reduce artifacts from autofluorescence and scattered light.

[36] http://tools.invitrogen.com/content.cfm?pageid=10935

Instrument parameters

Reader	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**PolarScreen**	**PolarScreen**	**PolarScreen**
Excitation wavelength	Green: 485 nm Red: 535 (25) nm Far Red: 610 (20) nm	Green: 485 nm Red: 535 (25) nm Far Red: 610 (20) nm	Green: 470 nm Red: 530 nm Far Red: 590nm
Emission wavelength	Green: 535 (25) nm Red: 590 (20) nm Far Red: 670 (40) nm	Green: 535 (25) nm Red: 590 (20) nm Far Red: 670 (40) nm	Green: 530 (20) nm Red: 590 (20) nm Far Red: 680 (20) nm
Flashes	10	10	20
Mirror	/	automatic	automatic
G-factor	calculated	calculated	calculated
Gain	optimal	optimal	optimal
Z-position	/	calculated	calculated
Settle time	0 ms	0 ms	0 ms

Support

Link

- Assay link: http://tools.invitrogen.com/content.fm?pageid=10935

Tecan Application Notes

- PolarScreen™ glucocorticoid receptor competitor assay, green/red
- PolarScreen™ Far Red Tyrosine Kinase Assay
- PolarScreen Red™ (Invitrogen) Glucocorticoid Receptor Assay

Transcreener®

Nucleotide (ADP, GDP, etc.) detection assay

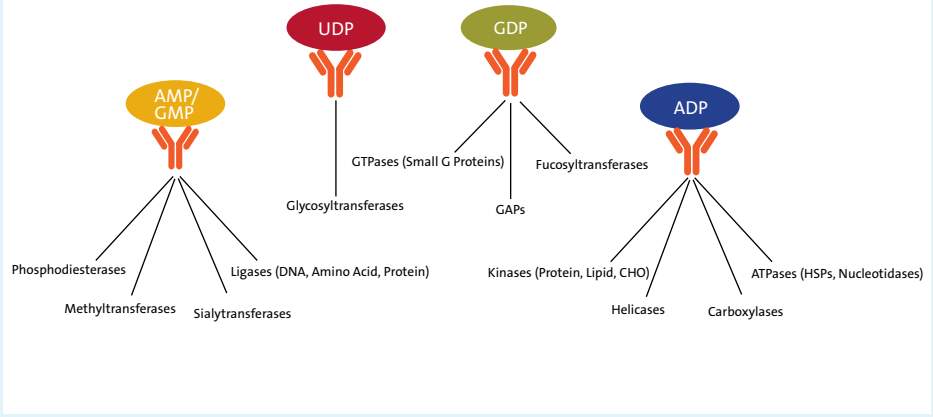


Figure 1: Transcreener targets

Assay	Readout
Transcreener ADP² Assays	FP, FI, TR-FRET
Transcreener AMP/GMP Assay	FP, FI
Transcreener GDP Assays	FP
Transcreener UDP Assays	FP

Table 1: Alternative readouts

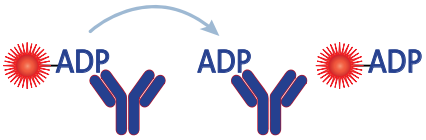


Figure 2: Transcreener principle

Assay overview

Technology
Fluorescence polarization (FP)
Fluorescence intensity (FI)
Time-resolved fluorescence resonance energy transfer (TR-FRET)

Principle, provider
Bellbrook's Transcreener assays[37] are designed to detect various mono- and dinucleotides using FP, TR-FRET or FI detection mode. Four assays (Table 1) cover thousands of target enzymes, including any kinase, ATPase or GTPase. Transcreener is a universal assay method that can be used across entire families of nucleotide-dependent enzymes. All assays are based on different antibodies that show a high affinity for one specific nucleotide (Figure 1).

Mechanism
The mechanism is the same for all Transcreener assays. The antibody is preloaded with the corresponding nucleotide, which is conjugated to a tracer molecule. All assays use a far red tracer that minimizes compound interference. For example, in the ADP² FP assay the detection mixture comprises of Alexa 633 ADP and a highly selective ADP monoclonal antibody. The Transcreener ADP² FP assay measures the progress of any enzyme that produces ADP by displacing the tracer by ADP thereby causing a decrease in fluorescence polarization. (Figure 2)

Support

Link
- Assay homepage: http://www.bellbrooklabs.com/transcreener_hts_assays.html

Tecan Application Notes
- Transcreener® ADP2 Fluorescence Polarization assay
- Homogeneous ADP Detection Using Transcreener® Technology on Tecan's Infinite® F500 and Infinite M1000 Multimode Microplate Readers

[37] http://www.bellbrooklabs.com/transcreener_hts_assays.html

Instrument parameters



Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**Transcreener FP**	**Transcreener FP**	**Transcreener FP**	**Transcreener FP**
Excitation wavelength	-	610(20) nm	610(20) nm	635(5) nm
Emission wavelength	-	670(25) nm	670(25) nm	680(20) nm
Flashes	-	10	10	10
Mirror	-	automatic	automatic	automatic
G-factor	-	calculated	calculated	calculated
Gain	-	optimal	optimal	optimal
Z-position	-	calculated	calculated	calculated
Settle time	-	50 ms	50 ms	50 ms

Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**Transcreener FI**	**Transcreener FI**	**Transcreener FI**	**Transcreener FI**
Excitation wavelength	585(9) nm	580(20) nm	580(20) nm	590(15) nm
Emission wavelength	627(20) nm	620(20) nm	620(20) nm	625(15) nm
Flashes	25	25-100	10	10
Mirror	-	automatic	automatic	-
Gain	optimal	optimal	optimal	optimal
Z-position	optimzed	-	-	calculated
Settle time	0 ms	0 ms	0 ms	0 ms
Flash frequency				100 Hz
Lag time	0 µs	0 µs	0 µs	-
Integration time	20 µs	20 µs	20 µs	20 µs

Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**Transcreener TR-FRET**	**Transcreener TR-FRET**	**Transcreener TR-FRET**	**Transcreener TR-FRET**
Measurement	-	Donor	Donor	Donor
Mode	-	FI top	FI top	FI top
Excitation wavelength	-	Eu: 320(25) nm Tb: 340(35) nm	Eu: 320(25) nm Tb: 340(35) nm	Eu: 317(20) nm Tb: 332(20) nm
Emission wavelength	-	620(10) nm	620(10) nm	620(10) nm
Lag time	-	150 µs	150 µs	60 µs
Integration time	-	500 µs	500 µs	500 µs
Flashes	-	50	10	125-165 (100 Hz)
Mirror	-	automatic	automatic	/
Gain	-	optimal	optimal	optimal
Z-position	-	-	calculated	calculated
Settle time	-	0 ms	0 ms	0 ms
Assay	**Transcreener TR-FRET**	**Transcreener TR-FRET**	**Transcreener TR-FRET**	**Transcreener TR-FRET**
Measurement	-	Acceptor	Acceptor	Acceptor
Mode	-	FI top	FI top	FI top
Excitation wavelength	-	Eu: 320(25) nm Tb: 340(35) nm	Eu: 320(25) nm Tb: 340(35) nm	Eu: 317(20) nm Tb: 332(20) nm
Emission wavelength	-	665(8) nm	665(8) nm	665(10) nm
Lag time	-	150 µs	150 µs	60 µs
Integration time	-	500 µs	500 µs	500 µs
Flashes	-	25	10	10 (100 Hz)
Mirror	-	automatic	automatic	/
Gain	-	optimal	optimal	optimal
Z-position	-	/	calculated	calculated
Settle time	-	0 ms	0 ms	0 ms

Alpha – Amplified Luminescent Proximity Homogeneous Assay

FRET equivalent with reduced distance limitations and amplification

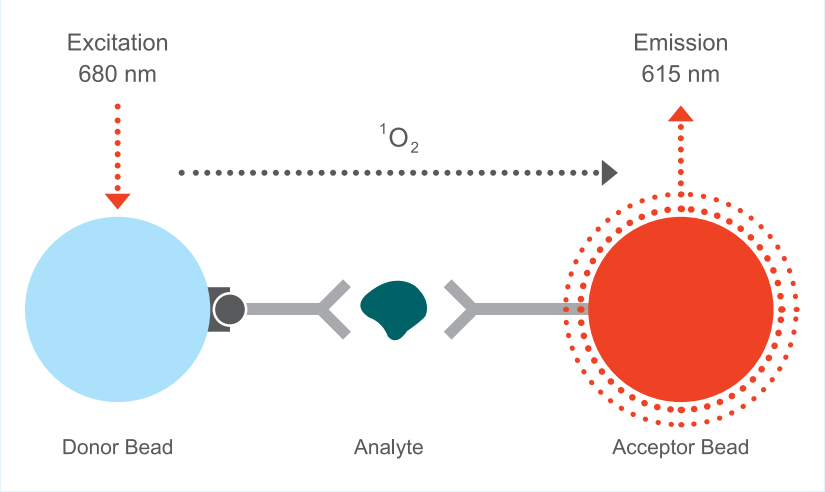


Figure 1: Schematic function of the Alpha technology demonstrated by AlphaLISA® assay.

Technology

Mechanism

Alpha[38] is a technology invented by PerkinElmer. The principle is similar to (TR-)FRET, since the Alpha technology also relies on the interaction of an acceptor and a donor to yield a signal (Figure 1). However, instead of using simple fluorophores, chemically reactive beads are used, and the chemistry that produces the signal is also different. The main advantage of AlphaScreen® and AlphaLISA® is that the distance between the interaction partners can be up to 200 nm, compared to 10 nm for (TR-)FRET. Furthermore, there is an amplification effect which increases the sensitivity of the assay. Background is reduced because the emission wavelength is lower than the excitation wavelength.

Assay design

The assay requires a donor bead, an acceptor bead, and a tagged antibody. The acceptor bead – coupled to a target-specific antibody – and the tagged antibody to capture the donor bead, are added to the sample and incubated to allow the components to bind to their target molecule. Finally, the donor bead is added, which binds to the tagged antibody.

When the donor bead is excited, it will convert ambient oxygen in its close environment (200 nm) into reactive oxygen molecule. If a binding event occurs, oxygen radicals will eventually reach the acceptor bead and cause an emission signal at a lower wavelength compared to the excitation wavelength.

The Alpha technology's versatility offers the possibility to assay many biological targets, including enzymes, receptor-ligand interactions, low affinity interactions, second messenger levels, DNA, RNA, proteins, protein-protein interactions, peptides, sugars and small molecules[38].

Major applications

- AlphaScreen / AlphaLISA

[38] http://www.perkinelmer.com/catalog/category/id/alphatech

Compatible readers

Features	Infinite F200 PRO	Infinite M1000 PRO
Temperature control	Ambient +5 °C to 42 °C	Ambient +4 °C to 42 °C
Shaking	Linear, orbital with variable amplitudes	Linear, orbital, double orbital with variable amplitudes
Injectors	2	2
Gas Control Module	Yes	No
Barcode reader	Yes	Yes

Typcal values: Amplified Luminescent Proximity Homogeneous Assay (Alpha)	Infinite F200 PRO	Infinite M1000 PRO
Light source	Xenon flash lamp	High power laser diode (680 nm) for high-end Alpha assays
Detector	Dedicated PMT for Alpha and fluorescence measurements	Low noise photon counting PCT (lumi PMT)
Sensitivity	≤50 ng/ml Omnibead	≤100 amol / well bio-LCK-P ≤2.5 ng/ml Omnibeads
Uniformity	≤ 5 % CV	≤ 3 % CV
Z'-Value	≥ 0.8	≥ 0.9
Reading speed	<11 min (384-well plate)	<2min (384-well plate)
Temperature correction	No	Yes
Filters	Optimized fluorescence filter	Optimized luminescence filter

Support

Links

- Assay homepage: http://www.perkinelmer.com/catalog/category/id/alphatech
- Alpha knowledge base: http://perkinelmerreagents.onconfluence.com/pages/viewpage.action?pageId=328672
- Nature advertisement: http://www.nature.com/nmeth/journal/v5/n12/pdf/nmeth.f.230.pdf

Tecan Application Notes

- Implementation of AlphaLISA® technology in the Infinite® M1000 PRO
- New Infinite® M1000 PRO with AlphaScreen® module
- Implementation of AlphaScreen® technology in the Infinite® M1000 PRO
- Implementation of AlphaScreen® technology on the Infinite® F200 PRO
- Implementation of AlphaLISA® technology on the Infinite® F200 PRO

AlphaScreen / AlphaLISA

Alpha Technology assay platforms

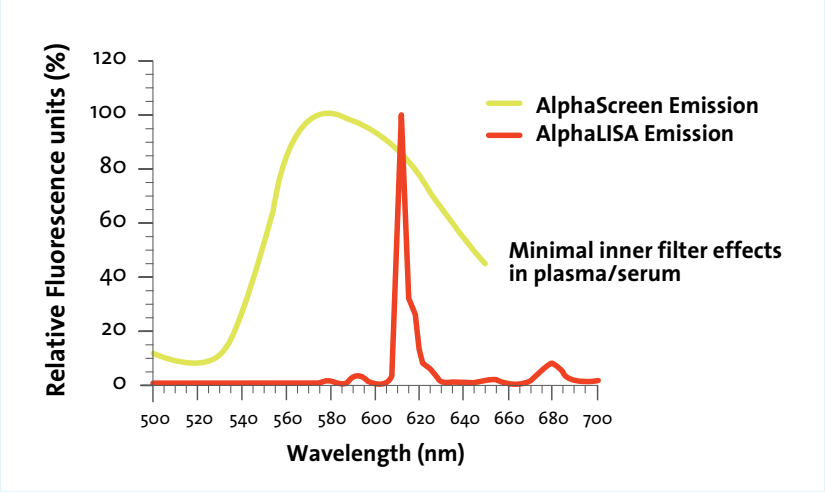


Figure 1: Emission spectra of TAR (AlphaScreen) and europium (AlphaLISA) acceptor beads.

Assay overview

Technology
Alpha Technology

Provider, Format
AlphaScreen and AlphaLISA are PerkinElmer's Alpha Technology[39] assay / reagent platforms. PerkinElmer supplies donor beads coupled to streptavidin, and blank acceptor beads for self-labeling. Additionally, acceptor beads can be purchased precoupled to antibodies specific for a broad range of targets.

Principle
AlphaLISA is a development targeting laboratories working with crude blood samples, as the autofluorescence of heme group overlaps with the emission peak (Figure 1) of the AlphaScreen acceptor. The main difference between AlphaLISA and AlphaScreen is that the AlphaLISA emission peak (europium emission) of the acceptor bead is smaller than that of the AlphaScreen assay (rubrene emission).

Mechanism
For a closer description of the assay mechanism, please refer to the Alpha Technology section.

Support

Links
- Tecan homepage: http://www.tecan.com/alphascreen
- Assay homepage: http://www.perkinelmer.com/catalog/category/id/alphatech
- Alpha knowledge base: http://perkinelmerreagents.onconfluence.com/pages/viewpage.action?pageId=328672
- PE recomended settings: http://perkinelmerreagents.onconfluence.com/pages/viewpage.action?pageId=328672#AlphaLISAAlphaScreennowashassays-Instrumentoptionssettings

Tecan Application Notes
- Implementation of AlphaLISA® technology in the Infinite® M1000 PRO
- New Infinite® M1000 PRO with AlphaScreen® module
- Implementation of AlphaScreen® technology in the Infinite® M1000 PRO
- Implementation of AlphaScreen® technology on the Infinite® F200 PRO
- Implementation of AlphaLISA® technology on the Infinite® F200 PRO

[39] http://www.perkinelmer.com/catalog/category/id/alphatech

Instrument parameters

Reader	Infinite M1000 PRO
Assay	**AlphaScreen**
Plate	White or gray plates
Excitation time	100 ms
Integration time	300 ms
Settle time	0 ms
Filter	AlphaScreen
Temperature correction	optional
Assay	**AlphaLISA**
Plate	White plates only
Excitation time	100 ms
Integration time	300 ms
Settle time	0 ms
Filter	AlphaLISA
Temperature correction	optional

Reader	Infinite F200 PRO
Assay	**AlphaScreen**
Plate	White or gray plates
Excitation wavelength	680 (30) nm
Emission wavelength	570 (100) nm
Excitation time	1000 ms
Integration time	500 ms
Gain	Calculated from well well with the highest signal or preoptimized and set manually
Settle time	0 ms
Assay	**AlphaLISA**
Plate	White plates only
Excitation wavelength	680 (30) nm
Emission wavelength	615 (20) nm
Excitation time	1000 ms
Integration time	500 ms
Gain	Calculated from well with the highest signal or preoptimized and set manually
Settle time	0 ms

LUMI-Luminescence

Light is emitted from the sample

Figure 1: Firefly

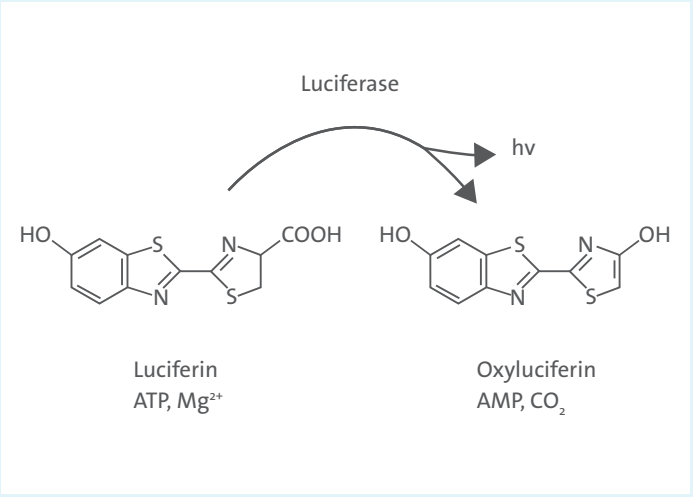


Figure 2: Substrate cleavage by luciferase

Technology

Luminescence is widely known as a reaction that causes the release of light. It can be caused by chemical reactions, electrical energy, subatomic motion, or stress on a crystal. For molecular biology, bioluminescence is the most important of the various luminescence reactions. At the core of this technology is the luciferase enzyme. Luciferases (Firefly, Renilla) (Figure 1) convert a substrate into an excited state (Figure 2). When returning to the ground state, a photon (light) is released (emitted).

One striking difference between fluorescence and luminescence is that luminescence requires no excitation light. This reduces the background to almost zero, resulting in outstanding sensitivity.

Various forms of luminescence can be differentiated.

1. Glow luminescence, which generates stable and measurable light up to several hours, for example the BioThema ATP assay
2. Flash luminescence, which is characterized by rapid, but short-lived, light generation, for example DLR and Aequorin (injectors are required for flash luminescence)
3. Multicolor luminescence, such as BRET$^{1/2/3/e}$ and Chroma-Glow™

Major applications

- Dual-Luciferase® Reporter Assay (DLR™)
- Bio Thema ATP detection kit
- BRET (Bioluminescence Resonance Energy Transfer)

Compatible readers




Features	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Reader design	Monochromator	Filter	Filter	Monochromator
Configuration	Quad4 Monochromators™, 2 excitation and 2 emission monochromators for high performance, high flexibility and accurate data acquisition	Up to 4 programmable filter pairs per slide that are easily ejected and exchanged through the front of the instrument	Up to 6 filter pairs per slide which are individually moveable, allowing any combination of Ex and Em filters; easy filter change, ID chip	Premium Quad4 Monochromators, 2 excitation and 2 emission monochromators allow for stray light reduction up to a factor of 10^7
DLReady™	Yes	Yes	Yes	Yes
Capability	Yes	Yes	Yes	Yes
Plate format	6- to 384-well plates NanoQuant Plate	6- to 384-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate
Temperature control	Ambient +5 °C to 42 °C	Ambient +5 °C to 42 °C	Ambient +4 °C to 42 °C	Ambient +4 °C to 42 °C
Injectors	2	2	2	2
Stacker	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	On-board stacker: stacks for 30 or 50 plates
Barcode reader	Yes	Yes	Yes	Yes

Typcal values: Luminescence (LUMI)	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Capability – glow	Yes	Yes	Yes	Yes
Capability – flash	Yes (with injectors)	Yes (with injectors)	Yes (with injectors)	Yes (with injectors)
Capability – scanning	No	No	No	Yes
Capability – multicolor (BRET)	Yes	Yes	Yes	Yes
Detector	Photon counting system with low dark current PMT	Photon counting system with low dark current PMT	Photon counting system with low dark current PMT	Photon counting low dark current PMT
Wavelength range	380 - 600 nm	380 - 600 nm	380 - 600 nm	380 - 600 nm
Sensitivity (flash)	12 amol ATP / well (218fM; 384-well plate) – 384 / 55 µl	12 amol ATP / well (218fM; 384-well plate) – 384 / 55 µl	12 amol ATP / well (218 fM; 384-well plate) – 384 / 55 µl	12 amol ATP / well (218 fM; 384-well plate) – 384 / 55 µl
Sensitivity (glow)	225 amol ATP / well (9pM; low volume 384-well plate)	225 amol ATP / well (9 pM; low volume 384-well plate)	225 amol ATP / well (9 pM; low volume 384-well plate)	225 amol ATP / well (9 pM; low volume 384-well plate)
Dynamic range	9 decades (OD2)	9 decades (OD2)	8 decades (OD1)	9 decades (OD2)

Support

Tecan Application Notes

- Dual-Luciferase® Reporter Gene Assay
- Gaseous luminescence standards on Infinite® multimode readers
- Peroxidase detection using SwordTM Peroxidase Reagents
- Human IL-6 chemiluminescent ELISA using Sword™ Peroxidase Reagents
- Emission spectra of luminescent signals
- An Optimized Mouse IL-6 ELISA using Sword Diagnostics Peroxidase Reagents (Infinite® M200)
- Luminescence sensitivity
- Mycoplasm detection with the MycoAlert™ assay system

Bio Thema ATP detection kit[42]

ATP detection kit based on luminescence

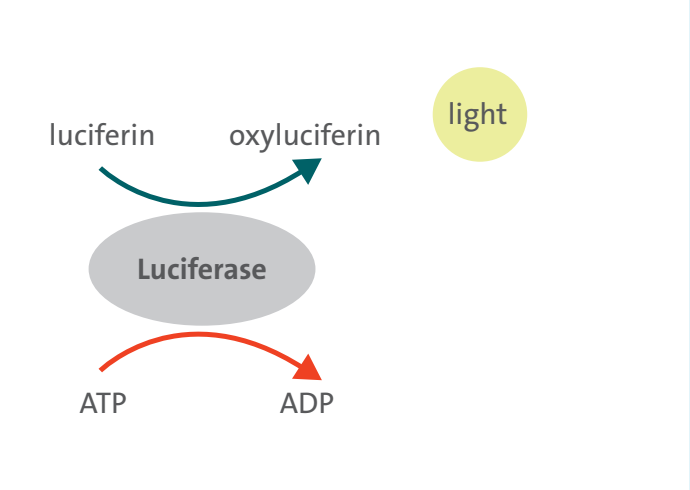


Figure 1: Schematic principle of luminescence caused by a luciferase based on the conversion of ATP.

Assay overview

Technology
Luminescence (Glow)

Principle, provider
ATP (Adenosine-Tri-Phosphate) is a molecule for short time energy storage and required for almost any reaction in living organisms. Biothema's ATP detection kit measure and quantifies the level of ATP and correlates it to the activity of an enzyme or cells.

Format
The kit consists of a luciferase, the substrate and an ATP standard to quantify the signal of the sample.

Mechanism
The luciferase and substrate are added to the sample. If ATP is present, the luciferase converts the substrate and light is released (Figure 1). If no ATP is present, no light is released. The more ATP is present in the sample the stronger is the signal. In a last step the signal is quantified by comparing it to a standard ATP curve.

Major applications
Major applications include ATP detection, Cell proliferation, Cytotoxicity, Enzymatic monitoring.

[42] http://biothema.se/products/kits /

Instrument parameters




Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**Bio Thema ATP**	**Bio Thema ATP**	**Bio Thema ATP**	**Bio Thema ATP**
Integration time	10,000 ms integration	10,000 ms integration	10,000 ms integration	10,000 ms integration
Attenuation	automatic	automatic	automatic	automatic
Settle time	-	0 ms	0 ms	0 ms

Support

Links
- Assay homepage: http://biothema.se/products/kits/

Tecan Application Notes
- Luminescence sensitivity

Dual-Luciferase® Reporter Assay (DLR™)[40]

Luminescence-based normalizable gene activator assay

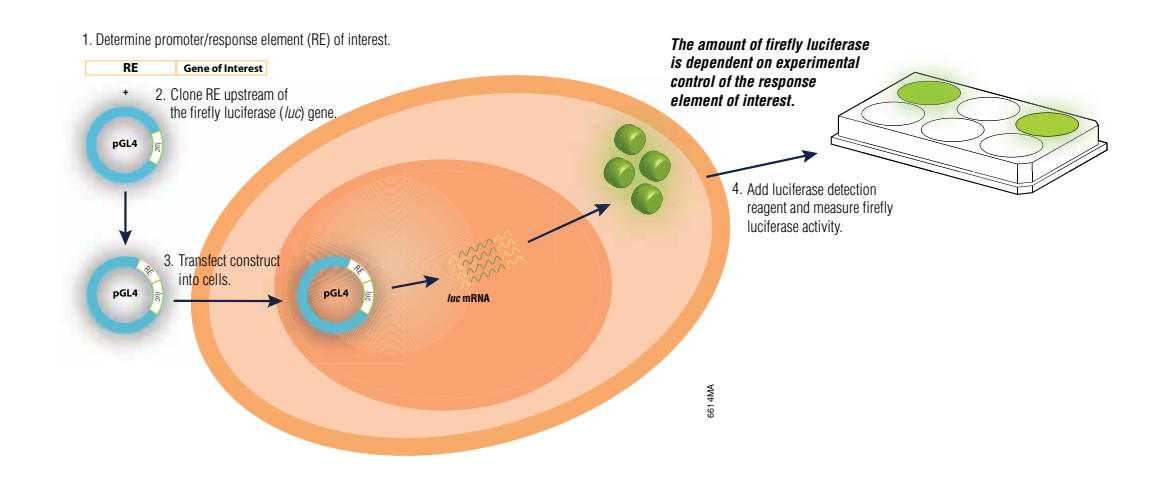


Figure 1: DLR assay mechanism

Assay overview

Technology

Luminescence (flash)

Purpose, provider, major application

Promega's DLR assay measures gene activation / expression using a luminescence-based readout. One particular challenge of gene activator assays is quantification of the result. The DLR assay resolves this issue by normalizing the output using two luciferases. While one luciferase measures the expression of the gene of interest, the second luciferase measures the expression of a housekeeping gene[41], which is used to normalize the signal. Promega has two series of firefly and Renilla luciferase vectors – pGL4 and pRL – designed for use with the DLR assay systems.

Mechanism

Cells need to be transfected with both luciferase reporter genes (Figure 1). The firefly luciferase reporter is measured first by adding Luciferase Assay Reagent II (LAR II) to generate a 'glow-type' luminescent signal. After quantifying the firefly luminescence, the reaction is quenched, and the Renilla luciferase reaction simultaneously initiated, by adding Stop & Glo® Reagent to the tube. The Stop & Glo Reagent produces a 'glow-type' signal from the Renilla luciferase, which decays slowly over the course of the measurement.

Assays are commonly measured over several hours, or even days. During this time period, the plate can either be shuttled between the incubator and the reader, or a temperature and gas controlled multimode reader such as the Infinite 200 PRO may be used.

[40] http://www.promega.com/products/reporter-assays-and-transfection/reporterassays/dual_luciferase-reporter-assay-system/
[41] Housekeeper genes are proteins or enzymes that are constitutionally expressed in most cells as for instance the DNA polymerase

Instrument parameters




Reader	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Assay	**DLR**	**DLR**	**DLR**	**DLR**
Well	Well wise	Well wise	Well wise	Well wise
Injector A	100 µl; refill for every injection	100 µl; refill for every injection	100 µl; refill for every injection	100 µl; refill for every injection
Wait	3 sec	3 sec	3 sec	3 sec
Measure	10,000 ms integration	10,000 ms integration	10,000 ms integration	10,000 ms integration
Attenuation	automatic	automatic	automatic	automatic
Injector B	100 µl; refill for every injection	100 µl; refill for every injection	100 µl; refill for every injection	100 µl; refill for every injection
Wait	3 sec	3 sec	3 sec	3 sec
Measure	10,000 ms integration	10,000 ms integration	10,000 ms integration	10,000 ms integration
Attenuation	automatic	automatic	automatic	automatic
Settle time	0 ms	0 ms	0 ms	0 ms

Support

Links
- Assay homepage: http://www.promega.com/products/reporter-assays-and-transfection/reporter-assays/dual_luciferasereporter-assay-system/

Tecan Application Notes
- Dual-Luciferase® Reporter Gene Assay

BRET (Bioluminescence Resonance Energy Transfer)

A FRET modification where the donor light comes from a luciferase reaction

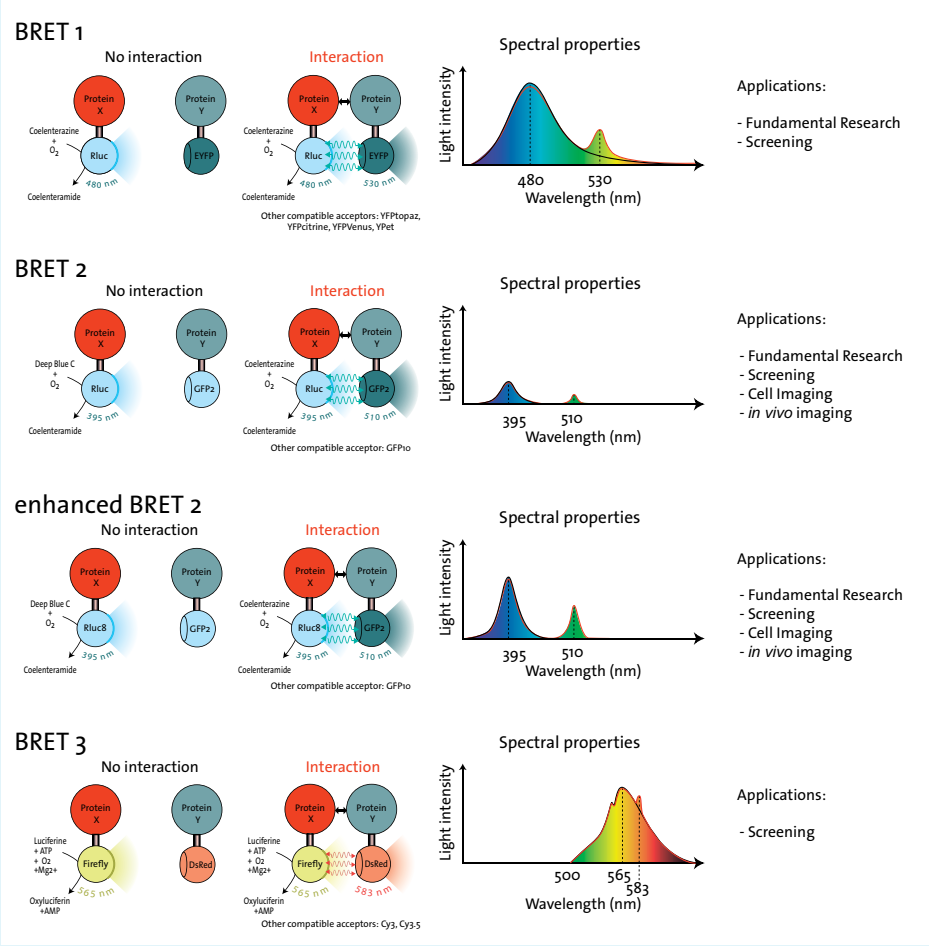


Figure 1: Summary of the most relevant BRET technologies.

Technology

BRET is a modification of FRET, the main difference being that the donor fluorophore is substituted by a luciferase. A major application for BRET is interaction studies. The advantage is that no excitation light is needed, and hence the background is much lower. BRET itself is a technology, and therefore can't be commercialized. However, various companies offer proprietary assay components for this technology, for example BRET[1/3/e] are an unlicensed technology, while BRET[2] is a licensed product of PerkinElmer. The difference between BRET[1] and BRET[2] lies in the selection of the donor and acceptor proteins / fluorophores (Figure 1). This results in a shift of the excitation and emission wavelength[42].

[42] Bacart J, Corbel C, Jockers R, Bach S, Couturier C: The BRET technology and its application to screening assays. Biotechnol J 2008, 3:311-324.

Compatible readers




Features	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Reader design	Monochromator	Filter	Filter	Monochromator
Configuration	Quad4 Monochromators™, 2 excitation and 2 emission monochromators for high performance, high flexibility and accurate data acquisition	Up to 4 programmable filter pairs per slide that are easily ejected and exchanged through the front of the instrument	Up to 6 filter pairs per slide which are individually moveable, allowing any combination of Ex and Em filters; easy filter change, ID chip	Premium Quad4 Monochromators, 2 excitation and 2 emission monochromators allow for stray light reduction up to a factor of 10^7
DLReady™	Yes	Yes	Yes	Yes
Capability	Yes	Yes	Yes	Yes
Plate format	6- to 384-well plates NanoQuant Plate	6- to 384-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate	6- to 1,536-well plates NanoQuant Plate
Temperature control	Ambient +5 °C to 42 °C	Ambient +5 °C to 42 °C	Ambient +4 °C to 42 °C	Ambient +4 °C to 42 °C
Injectors	2	2	2	2
Stacker	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	Connect: stacks for 30 or 50 plates	On-board stacker: stacks for 30 or 50 plates
Barcode reader	Yes	Yes	Yes	Yes

Typcal values: Luminescence (LUMI)	Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Capability – glow	Yes	Yes	Yes	Yes
Capability – flash	Yes (with injectors)	Yes (with injectors)	Yes (with injectors)	Yes (with injectors)
Capability – scanning	No	No	No	Yes
Capability – multicolor (BRET)	Yes	Yes	Yes	Yes
Detector	Photon counting system with low dark current PMT	Photon counting system with low dark current PMT	Photon counting system with low dark current PMT	Photon counting low dark current PMT
Wavelength range	380 - 600 nm	380 - 600 nm	380 - 600 nm	380 - 600 nm
Sensitivity (flash)	12 amol ATP / well (218fM; 384-well plate) – 384 / 55 µl	12 amol ATP / well (218fM; 384-well plate) – 384 / 55 µl	12 amol ATP / well (218 fM; 384-well plate) – 384 / 55 µl	12 amol ATP / well (218 fM; 384-well plate) – 384 / 55 µl
Sensitivity (glow)	225 amol ATP / well (9pM; low volume 384-well plate)	225 amol ATP / well (9 pM; low volume 384-well plate)	225 amol ATP / well (9 pM; low volume 384-well plate)	225 amol ATP / well (9 pM; low volume 384-well plate)
Dynamic range	9 decades (OD2)	9 decades (OD2)	8 decades (OD1)	9 decades (OD2)

Support

Tecan Application Notes

- Bioluminescence Resonance Energy Transfer (BRET)
- Optimization of BRET²™ measurement parameters

Technical terms

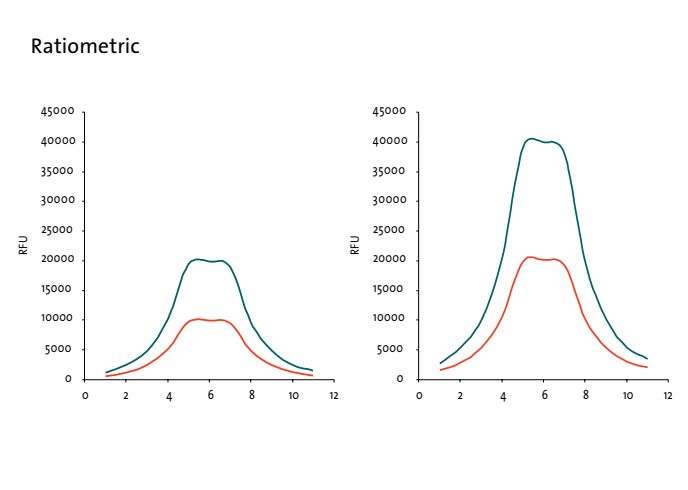


Figure 1: Two assay signals from the same sample. The difference in intensity is caused by varying amounts of added assay reagents

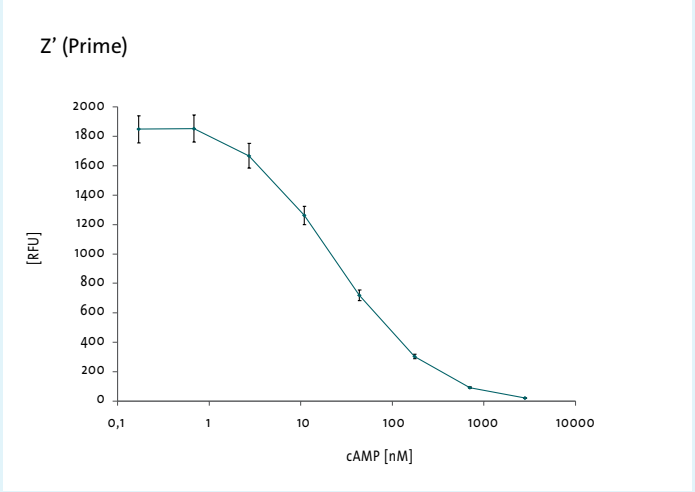


Figure 2: Data acquisition for Z' calculation examplified by a cAMP assay.

Homogeneous
The term homogenous refers to the design of an assay, and means that no washing is required. All the required assay components are added either simultaneously or one after another, but there are no washing steps in between. This simplifies the assay set-up.

Ratiometric
Signal artifacts can have many different reasons. One cause are concentration differences of assay reagents (e.g. due to pipetting mistakes). Ratiometric assays resolve this issue by normalizing the signal to the concentration of the assay reagents for each sample separately.

For example:
Some TR-FRET assays are excited at 320 nm (Eu) and emission is measured at 620 nm and 665 nm.

The **emission signal measured at 620 nm** (donor) is independent of the interaction, depending solely on the concentration of the added assay reagents, and is therefore used as an internal reference.
The **emission signal at 665 nm** (acceptor) is used as an indicator of the suspected biological interaction being assessed, and represents the amount of interacting molecules.

To eliminate the influence of the reagent concentration, the emission signal at 665 nm is divided by the signal measured at 620 nm, eliminating well-to-well deviations.
Ratiometric measurements include FP, FRET, TR-FRET and BRET-based assays.

G-Factor
G ... Geraete (ger.) / Device Factor (eng.)
Filters, mirrors and quartz fibers absorb light, resulting in signal intensity losses. However, the amount of light lost depends on the polarization angle of the light. To compensate for these losses, a correction factor (G-factor) needs to be calculated. In general, it should be between 0.6 and 1.4 .

Z' (Prime)
Z' is a quality criterion which tries to quantify the suitability of an assay or instrument feature for a certain application. It is a dimensionless factor which considers the standard deviations (SD) and means (μ) of both, the positive (p) and negative (n) controls.[1]
In general, Z' may be between 0 and 1 but wold ideally be close to 1.

$$Z' = 1 - \frac{3 \times (SD_p + SD_n)}{\mu_p - \mu_n}$$

[1] BZhang JH, Chung TDY, Oldenburg KR (1999). "A simple statistical parameter for use in evaluation and validation of high throughput screening assays". Journal of Biomolecular Screening 4: 67–73. doi:10.1177/108705719900400206. PMID 10838414.

Appendix

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Reader-Assay Compatibility, validations (V) and certifications (C)

			Infinite M200 PRO	Infinite F200 PRO	Infinite F500	Infinite M1000 PRO
Technology	Reader capability	**ABS**	x	x	x	x
	Reader capability	**FI**	x	x	x	x
	Reader capability	**TRF**	x	x	x	x
	Reader capability	**FRET**	x	x	x	x
	Reader capability	**TR-FRET**		x	x	x
	Reader capability	**FP**		x	x	x
	Reader capability	**ALPHA**		x		x
	Reader capability	**LUMI**	x	x	x	x
Binding studies	BRET	**LUMI**	x	x	x	x
	HTRF®	**TR-FRET**		x white plates	x (C)	x white plates
	Delfia®	**TRF**	x	x	x	x
	Omnia®	**FI**	x	x	x (V)	x (V)
	Predictor™ hERG	**FP**		x	x (V)	x (V)
Gene expression	GeneBLAzer™	**FRET**	x (V)	x (V)	x (V)	x (V)
	DLR™	**LUMI**	x (C)	x (C)	x (C)	x (C)
Signal transduction	Z-Lyte®	**FRET**	x	x	x (V)	x (V)
	LanthaScreen™	**TR-FRET**		x (C)	x (C)	x (C)
	PolarScreen™	**FP**		x	x (V)	x (V)
	Adapta®	**TR-FRET**		x	x (V)	x (V)
	Transcreener ADP2	**FP**		x	x (C)	x (C)
	Transcreener ADP2	**FI**	x (C)	x (C)	x (C)	x (C)
	Transcreener ADP2	**TR-FRET**		x	x (V)	x (V)

Tecan – Who we are

Tecan is a leading global provider of life science laboratory instruments for the biopharmaceuticals, forensics, clinical diagnostics and academic sectors, specializing in the development and production of automation and detection solutions, including microplate readers, microarray products and washers.

Founded in Switzerland in 1980, Tecan has manufacturing, research and development sites in both North America and Europe, and maintains a sales and service network in 52 countries. To date, Tecan has distributed approximately 20,000 microplate readers worldwide, and is committed to continuous technological improvements and compliance with the highest global quality standards.

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