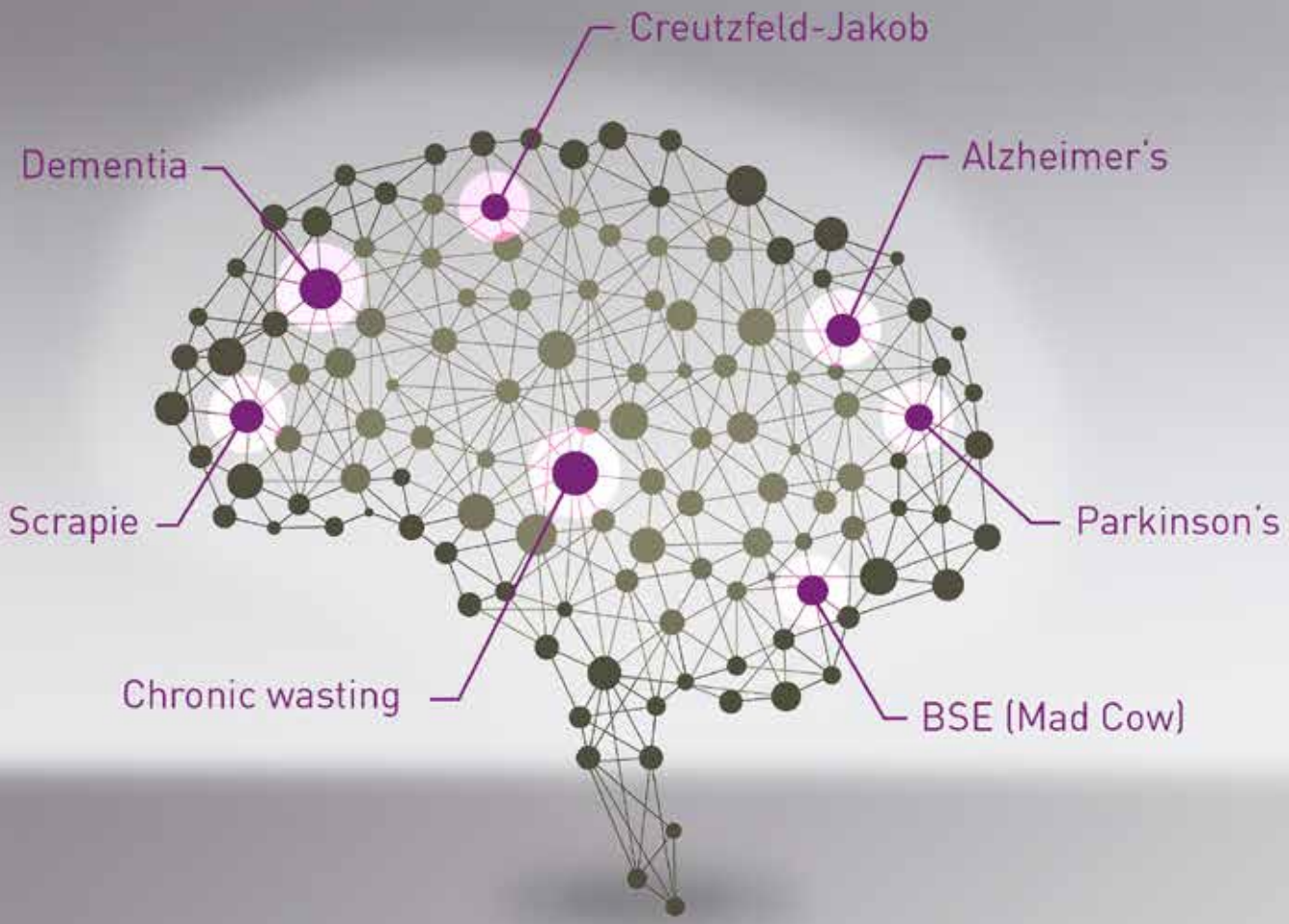
The best Microplate Reader technology for neuro-degenerative disease research



BMG LABTECH

The Microplate Reader Company

Performance and flexibility for neuro-degenerative disease research

BMG LABTECH´s multi-mode microplate readers represent the best combination of performance and flexibility for all of your life science and R&D applications. With their ability to capture fast, full UV/vis absorbance spectra, to monitor rapid and slow kinetic reactions, and to perform fluorescence intensity (incl. FRET), luminescence (incl. BRET), TR-FRET and AlphaScreen®/AlphaLISA® detection, the CLARIOstar® and FLUOstar® Omega fulfil all research needs.

Top and bottom plate reading, multi-colour detection, well scanning, precise temperature control, multi-mode shaking, all enhance assay flexibility. Moreover, the addition of on-board "smart" injectors provides the ability to dispense reagents and initiate kinetic reactions.

CLARIOstar®

The CLARIOstar is a multi-mode, high-performance microplate reader that combines the flexibility of monochromators with the sensitivity of filters. Thanks to BMG LABTECH's proprietary LVF Monochromators™, it is the most sensitive monochromator-based reader on the market. The ideal microplate reader for assay development, it provides the highest levels of flexibility and sensitivity for all fluorescence intensity and TR-FRET-based assays. The CLARIOstar reads all plate formats from 6- to 1536-well in all detection modes and can be equipped with an Atmospheric Control Unit (ACU) for live cell-based assays.

FLUOstar® Omega

A filter-based microplate reader, the FLUOstar® Omega represents the best combination of performance, flexibility, and value for money for all of your applications.

Thanks to its robustness and precision, it was chosen by Rocky Mountain Labs, Montana, USA, as the reference reader for the development of the RT-QuIC assay (Wilham et al., 2010).

Fully modular, the instrument can at any time be upgraded to a fully-equipped reader with up to six detection modes, if additional features or detection modes are needed in the future.

The FLUOstar® Omega reads all plate formats from 6- to 1536-well in absorbance and up to 384-well in all other detection modes.

Advanced Time-Resolved Fluorescence

The CLARIOstar has excellent TRF and TR-FRET sensitivity. Assays such as HTRF®, LANCE®, Delfia®, and LanthaScreen® can now be performed with outstanding performance. The reader was certified for both white and black plates by Cisbio for its HTRF-based assays (e.g.: tau aggregation assay).

For enhanced TRF capabilities, the FLUOstar Omega utilizes an advanced optic head for TRF and TR-FRET detection.

With a high intensity xenon flash lamp, assay-optimized filters and adjustable gain, both readers outperform any microplate reader in their respective class.

Seeding assays

The FLUOstar Omega is the most used and popular platform dedicated to the detection of prion and amyloid seeding assays in the microplate format. It provides robustness and the ability to withstand extensive shaking, even for long kinetics.

The CLARIOstar extends these features offering greater flexibility and sensitivity for assay development and a broader range of assays.

Both instruments offer the following dedicated features for seeding assays:

- High-quality German engineering and manufacturing for higher robustness and functionality
- Shaking and incubation over long periods of time (20-68 hours)
- Ability to withstand prolonged and continuous high-speed shaking
- Dedicated plate carrier for highest microplate stability even in thorough shaking conditions
- Linear, orbital, and double-orbital shaking
- Incubation up to 45°C or 65°C
- Top and bottom reading
- Data collection without interruption and output to BMG LABTECH's MARS data analysis software and/or Excel®
- Dedicated multi-user Control and MARS data analysis software

Selected references

Prion seeding assays:

- Wilham JM et al., *PLoS Pathog.,* 2010: Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays
- Orrú et. al., *N. Engl. J. Med.,* 2014: A test for Creutzfeld-Jakob disease using nasal brushings
- McGuire et al., Ann Neurol. 2016: Cerebrospinal fluid real-time quaking-induced conversion is a robust and reliable test for sporadic Creutzfeldt-Jakob disease: An international study

β-amyloid fibril formation:

- Nasir et al., ACS Chem Neurosci. 2015: Fluorescent Filter-Trap Assay for Amyloid Fibril Formation Kinetics in Complex Solutions
- Habchi et al., PNAS, 2017: Systematic development of small molecules to inhibit specific microscopic steps of Aβ42 aggregation in Alzheimer's disease

Tau-aggregation:

- Polanco et al., J Biol Chem, 2016: Extracellular Vesicles Isolated from the Brains of rTg4510 Mice Seed Tau Protein Aggregation in a Threshold-dependent Manner*

α-synuclein aggregation:

- Buell et al., PNAS 2014: Solution conditions determine the relative importance of nucleation and growth processes in α-synuclein aggregation
- Grey et al., J Biol Chem, 2015: Acceleration of α-Synuclein Aggregation by Exosomes
- Brown et al., Sci Rep. 2016: α-Synuclein suppresses both the initiation and amplification steps of α-synuclein aggregation via competitive binding to surfaces*

*For these studies the CLARIOstar was employed

Following Abeta fibrillization/aggregation in real-time using a FLUOstar Omega

Frank Baumann, Hertie Institute for Clinical Brain Research, Tübingen, Germany

Introduction

Aggregation of the amyloid-β (Aβ) peptide is a fundamental hallmark for Alzheimer's disease. The formation of extracellular senile plaques will lead to synaptic and neuronal damages in clinical demented patients. The aggregation process of Aβ peptide is seen as seed driven. These seeds consist of small stable aggregates of Aβ. It is thought that these aggregates are already present in early stages of Alzheimer's even before a patient experiences any symptoms. If this is true, determination of these early aggregates (aggregation seeds) would be an excellent diagnostic tool.
Here we present a cell-free assay that allows determination of the amount of aggregation seeds from brain tissue homogenates. The assay is run over 2-3 days using the FLUOstar Omega microplate reader from BMG LABTECH.

Assay Principle

The assay uses Thioflavin T to follow the amyloid formation (Fig. 1). Thioflavin T is a benzothiazole salt that is known to show increased fluorescence when bound to beta sheet-rich structures, such as in amyloid fibrils of Aβ.

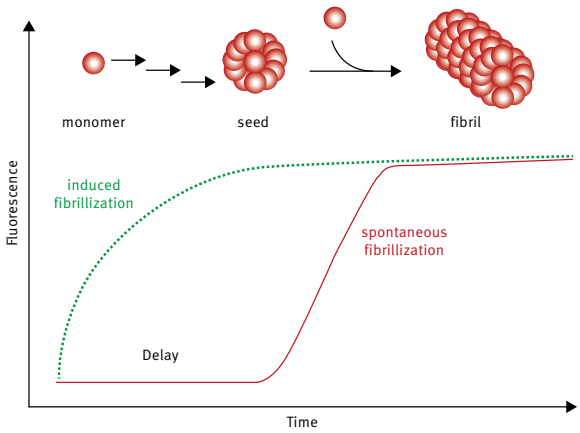


Fig. 1: Fibrillization process followed over time

Before aggregation or fibrillization can start a critical amount of initial aggregation seeds need to be present or spontaneously formed. This is a thermodynamically unfavoured process even in the presence of excess monomeric Aβ, kinetically slow and results in a delay in time before measurable aggregation starts. Once enough seeds have formed a massive and steep increase of fibrillization can be monitored by following the increase of ThT fluorescence due to incorporation into newly formed fibrils.

After some time a plateau is reached indicating the end of the reaction. The delay time is significant and can be shortened by exogenous addition of aggregation seeds. These seeds accelerate the increase in fluorescence in relation to their amount.

Results & Discussion

The time until the signal starts to increase is the lag time. The MARS data analysis software offers the possibility to create 4-parameter fits of the signal curves from which the lag times are calculated (lag times correspond to the EC20 value of the fit). Initial fibril seeds are formed until the lag time is reached. Considering this, the lag time can be used as a measure to compare different brain homogenates (Fig. 2).

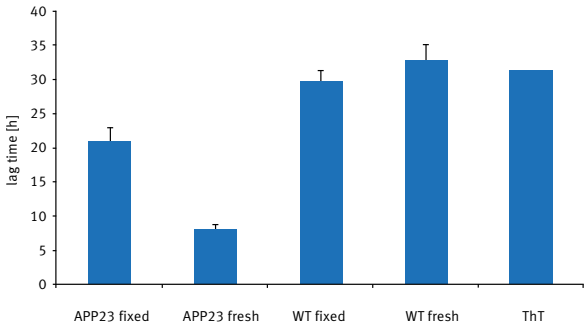


Fig. 2: Lag times of samples containing mice brain homogenates. Fixed and fresh frozen transgenic (APP23) and WT mice are compared. As a control thioflavin T only (ThT) was measured on the same microplate (n=1). Error bars refer to 3 biological replicates.

From figure 2 it can be followed that the lag times of the wild type are bigger compared to the lag times obtained for the tg mice. Further a difference can be seen between fixed and fresh-frozen APP23 samples. As expected the fresh samples induce Aβ deposition much faster. Nonetheless, the fixed APP23 samples show compared to the WT a significantly lower lag time indicating that fixation in formaldehyde is not sufficient to prevent Aβ aggregation.

Conclusion

With the help of the FLUOstar Omega microplate reader it is possible to prove that the in vitro assay is reliable to detect seeding activity in brain samples. In addition it allows quantitative comparison of seeding activity which with only little effort can be statistically validated.

Real-Time Quaking Induced Conversion Assay for Prion Seeding

Maggie Nakamura, BMG LABTECH

Introduction

Prions are transmittable pathogens that cause an abnormal folding of a brain protein in both humans and animals. Infection results in brain damage and is fatal. Some examples of these neurodegenerative diseases are Scrapie, Bovine Spongiform Encephalopathy, and Creutzfeldt-Jakob Disease. Previously, prions were studied using lengthy bioassays where infected animals were studied over long periods of time (1-6 months). This was both time consuming and costly to maintain the infected animal. Researchers at Rocky Mountain Laboratories in Hamilton, Montana have developed a prion seeding assay called Real-Time Quaking Induced Conversion Assay (RT-QuIC) that gives end point quantitation for measuring the levels of prions in infected samples. This assay is faster and yields higher throughput compared to previous methods. The assay can be completed in as few as 20 hours and is as sensitive, if not more so, as whole animal models.

Assay Principle

Combining parts of the original Quaking Induced Conversion (QuIC) assay and the amyloid seeding assay (ASA), the RT-QuIC assay is used to estimate the relative amount of prion seeding. The assay measures serial dilutions of samples, statistically estimating the seeding dose (SD). Very small amounts of infectious prions are added to normal prion protein to seed or cause the misfolding of the prion proteins as seen in the disease. The assay is quantitated by measuring serial dilutions of the samples and determining the loss of seeding activity, which is the end point dilution.

The fluorescent dye thioflavin T (ThT) is used as a prion seeding marker. When ThT is added to recombinant prion proteins, it becomes incorporated when polymerization occurs causing an increase in fluorescence over time.

BMG LABTECH's Omega series of readers have the ability to shake and incubate microplates over long periods of time. A POLARstar Omega was used to measure RT-QuIC samples every 15 minutes for 20-68 hours while alternately shaking and resting for a minute.

Results and Discussion

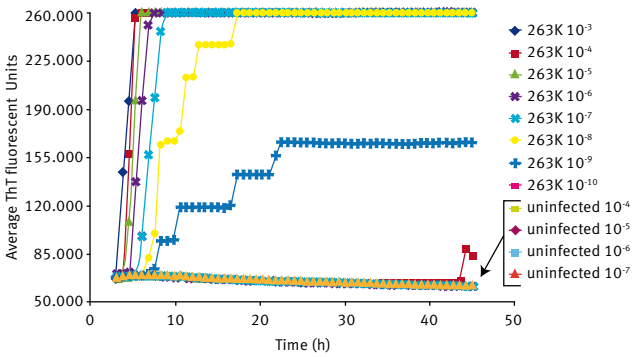


Fig. 1: RT-QuIC sensitivity: analysis of dilutions of a scrapie hamster brain homogenate stock 263K 80 - 85 dags post infection (DPI).

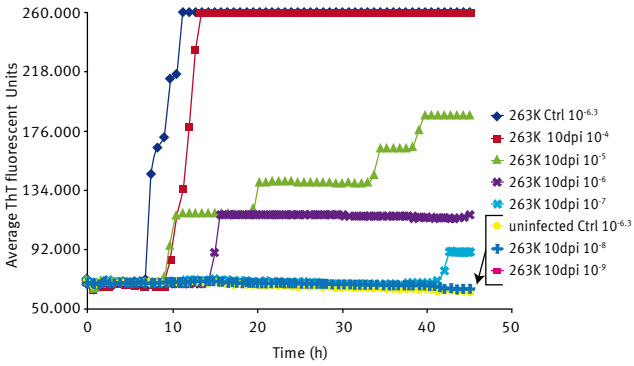


Fig. 2: RT-QuIC end-point dilution analysis of three 263K-inoculated preclinical 10 days post injection hamster BHs.

The SD50/gram of tissue for the 85 DPI samples (10E12) was higher than the 10 DPI (10E8.2) because it had a longer time for onset.

Conclusion

Prion seeding can be measured faster and in a higher throughput using the RT-QuIC assay and a microplate reader. Some of the transmissible spongiform encephalopathies that have been shown to work using RT-QuIC include hamster and sheep scrapie, deer chronic wasting disease, Creutzfeldt-Jakob Disease (CJD), and Bovine Spongiform Encephalopathy (BSE). The Omega series of plate readers from BMG LABTECH are both functional and robust to withstand the many days of shaking at high speeds required for this assay.

Detection of human tau protein aggregation

Delphine Jaga, Cisbio Bioassays, France

Introduction

The tau protein stabilizes microtubule structures in the brain. These structures are supporting the nutrient transport between neurons. Abnormal tau protein leads to collapse of structure and transport – plaques will be developed. This happens in patients that undergo neurodegeneration, e.g. in Alzheimer´s. The level of a patient´s tau protein can therefore be an indicator of a neurodegeneration disease state. To that end Cisbio developed a tau aggregation kit (cat. no.: 6FTAUPEG) that can be applied to cell cultures, brain tissue extracts, and recombinant proteins.

Assay Principle

Tau aggregates are measured using a sandwich immuno-assay, using an anti-tau monoclonal antibody labelled either with terbium-cryptate or d2, ensuring assay quality reproducibility and signal quality. The specific HTRF signal that is generated is proportional to the tau aggregates.

Fig. 1: : HTRF® tau aggregation assay principle.

Data processing including Ratio and DeltaF % calculations is supported by predefined software templates that come for free with every instrument.

Results and Discussion

Assay specificity and linearity

Figure 2 shows that the assay (measured on a PHERAstar *FS* microplate reader) can clearly distinguish between samples containing tau chemically aggregated and samples containing non aggregated tau.

While non aggregated tau does not show a significant increase, HTRF values of aggregated tau samples increase with concentration. There is a linear relationship for tau aggregation.

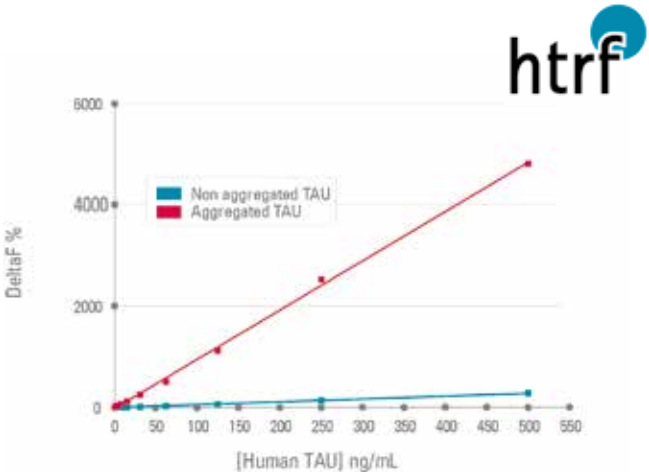


Fig. 2: DeltaF% values obtained for aggregated tau and non aggregated tau.

Tau aggregation on transgenic mouse brain extracts

Tau aggregation was determined in brain extract samples from transgenic mice (Tau/PSEN2/APP). From these mice it is known that they develop neurodegenerative pathology over time (Fig. 3).

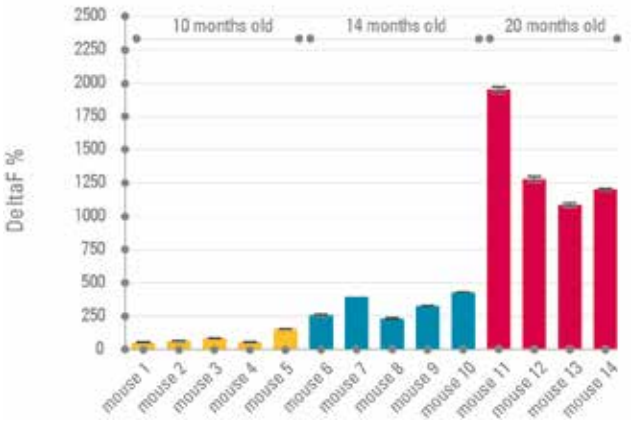


Fig. 3: Monitoring of tau aggregation over 20 months.

The figure shows that not only tau aggregates in late stages can be detected with the assay (red bars). The assay is specific enough to discriminate between early stages of tau fibrillization (yellow and blue bars).

Conclusion

The tau aggregation assay in combination with the PHERAstar *FS* microplate reader enables:

- Detection and quantification of tau aggregates in brain tissue and screening of tau modulators
- Kinetic of tau aggregation and dissociation
- The use of small sample size < 10 µl

FLUOstar® Omega - Technical specifications

Due to the modularity of BMG LABTECH's instruments, all or combinations of the features below can be installed at purchase or upgraded at any time. Please contact your local representative for more details or a quote.

Detection modes	Fluorescence intensity - including FRET UV/vis absorbance Luminescence (flash and glow) - including BRET Time-Resolved Fluorescence - including TR-FRET AlphaScreen®/AlphaLISA®	
Measurement modes	Top and bottom reading Endpoint and kinetic Sequential multi-excitation Sequential multi-emission Ratiometric measurements Well scanning	
Microplate formats	Up to 384-well plates, 1536-well plates in absorbance, user-definable	
Light source	High energy xenon flash lamp	
Detectors	Side window photomultiplier tube	
Optical filters	Excitation and emission filter wheels for 8 filters each	
Spectral range	240 - 740 nm or 240 - 900 nm Absorbance spectrometer: 220 - 1000 nm	
Sensitivity	FI	< 0.2 fmol/well fluorescein
	TRF	< 30 amol/well europium
	High-End TRF for Omega	< 3 amol/well europium
	LUM	20 amol/well ATP DLReady™ certified
	AlphaScreen®	< 100 amol* (384)
	Abs with spectrometer	Spectral range: 220 - 1000 nm Full spectrum captured in < 1 s/well Selectable spectral resolution: 1 - 10 nm OD range: 0 to 4 OD Accuracy: < 1% at 2 OD Precision: < 0.5% at 1 OD and < 0.8% at 2 OD
Read times	Flying mode: 9 s (96), 16 s (384)	
Reagent injection	Up to 2 built-in reagent injectors Injection at measurement position (6 to 384-well) Individual injection volumes for each well (3 to 500 µL) Variable injection speed up to 420 µL/s Up to four injection events per well Reagent back flushing	
Shaking	Linear, orbital, and double-orbital with user-definable time and speed	
Gas vent	System to inject an atmosphere or to pull a vacuum into the reader	
Incubation	+5°C above ambient up to 45°C or 65°C	
Software	Multi-user software package including Reader Control and MARS data analysis software	
Dimensions	Width: 44 cm, depth: 48 cm, height: 30 cm; weight: 28 kg	
Accessories		
Stacker	Plate handler for up to 50 microplates - continuous loading feature	
THERMOstar	Microplate incubator and shaker	
LVis Plate	Microplate designed to measure 16 low volume (2 µL) samples and standard cuvettes. Incorporating NIST filters and holmium oxide standards for instrument performance test. Sensitivity: 2 ng/µL dsDNA	
Filters	Optimized for dyes, fluorophores and specific assays Filters for all applications from UV to NIR Customized filters available upon request	
Upgrades	Upgrades to include options such as additional detection modes, reagent injectors, extended temperature control, etc. are available. Please contact your local representative for more information.	

* Limit of detection < 100 amol of biotinylated and phosphorylated polypeptide (P-Tyr-100 assay kit, PerkinElmer, #6760620C), measured in white 384 small volume microplates (17 µL/well)

Limit of detection was calculated according to the IUPAC standard: $3x(SD_{blank})/slope$

CLARIOstar - Technical specifications

Due to the modularity of BMG LABTECH's instruments, all or combinations of the features below can be installed at purchase or upgraded at any time. Please contact your local representative for more details or a quote.

Detection modes	Fluorescence intensity - including FRET Luminescence (flash and glow) - including BRET UV/vis absorbance Fluorescence polarization / Anisotropy Time-Resolved Fluorescence - including TR-FRET AlphaScreen® / AlphaLISA® / AlphaPlex™	
Measurement modes	Top and bottom reading Endpoint and kinetic measurements Spectral scanning (fluorescence, luminescence and absorbance) Well scanning	
Microplate formats	6- to 1536-well plates, LVis Plate with 16 microspots (2 µL)	
Light sources	High energy xenon flash lamp Dedicated laser for AlphaScreen® / AlphaLISA®/AlphaPlex™	
Detectors	Low-noise Photomultiplier Tube (PMT) UV/vis absorbance spectrometer	
Dual LVF Monochromator™	Fluorescence, luminescence: top and bottom Fluorescence excitation / emission spectral scanning Luminescence emission spectral scanning Spectral range: 320 - 850 nm (selectable increments from 0.1 to 10 nm) Software selectable bandwidths: 8 - 100 nm	
Linear Variable Dichroic Mirror	Spectral range: 340 - 740 nm (selectable increments of 0.1 nm)	
UV/vis absorbance spectrometer	Spectral scanning or up to 8 discrete wavelengths in less than 1 sec / well Spectral range: 220 - 1000 nm (selectable increments from 1 to 10 nm) Bandwidth: 3 nm	
Optical filters	Top and bottom for all detection modes, except absorbance Up to 4 excitation filters, 4 emission filters, and 3 dichroic mirrors Spectral range: 240 - 900 nm	
Sensitivity*	FI LVF Monochromator	Top: < 0.35 pM fluorescein, 384sv, 20 µL (< 7 amol/well) Bottom: < 3.0 pM fluorescein, 384, 50 µL (< 150 amol/well)
	FI filters	Top: < 0.15 pM fluorescein, 384sv, 20 µL (< 3 amol/well) Bottom: < 1.0 pM fluorescein, 384, 50 µL (< 50 amol/well)
	FP	< 0.5 mP SD at 1 nM fluorescein, 384sv, 20 µL
	TRF	< 20 fM europium, 384, 80 µL
	HTRF®	HTRF® certified for black and white microplates Reader Control Kit (Eu) after 18h incubation, 384sv, 20 µL > 880 % Delta F for High Calibrator > 30 % Delta F for Low Calibrator < 2.0 % CV for Standard 0
	LUM	< 0.4 pM ATP, 384sv, 20 µL (< 8 amol/well) Dynamic range: 9 decades
	AlphaScreen®	< 100 amol/well P-Tyr100 (384sv, 20 µL)
	Abs with spectrometer	Accuracy: < 1% at 2 OD Precision: < 0.5% at 1 OD and < 0.8% at 2 OD Dynamic Range: 0 - 4 OD
Read times	1 flash: 8 s (96) 15 s (384) 28 s (1536) 10 flashes: 19s (96) 57 s (384) 184 s (1536)	
Reagent injection	Up to 2 built-in reagent injectors with reagent back flushing Individual injection volumes for each well 3 to 500 µL (optional up to 2 mL) Variable injection speed up to 420 µL/s	
Shaking	Linear, orbital, and double-orbital with user-definable time and speed	
Incubation	+3°C above ambient to 45°C (65°C optional)	
Software	Integrated fluorophore library Multi-user software package including Reader Control and MARS data analysis software, FDA 21 CFR Part 11 compliant	
Dimensions	Width: 45 cm, depth: 51 cm, height: 40 cm; weight: 32 kg	
Accessories		
ACU	Actively regulates O_2 and CO_2: 0.1 - 20%	
LVis Plate	Measure 16 low-volume samples (2 µL) and QC standards	
Stacker	Magazines for up to 50 plates - continuous loading feature	

BMG LABTECH's LVF Monochromator includes technology covered under US Patent 6,700,690, for which BMG LABTECH has an exclusive license for the microplate reader market.
*Limit of detection (sensitivity) was calculated according to the IUPAC standard: $3x(SD_{blank})$ / slope
Specifications are subject to change without notice.

BMG LABTECH

Headquarters Germany
BMG LABTECH GmbH
Allmendgrün 8
77799 Ortenberg
Tel. +49 781 96968 -0
sales@bmglabtech.com

Australia
BMG LABTECH Pty. Ltd.
2/24 Carbine Way
Mornington, Victoria, 3931
Tel. +61 3 5973 4744
australia@bmglabtech.com

France
BMG LABTECH SARL
7, Rue Roland Martin
94500 Champigny s/Marne
Tel. +33 1 48 86 20 20
france@bmglabtech.com

Japan
BMG LABTECH JAPAN Ltd.
1-6-2, Shimo-cho
Omiya-ku
330-0844 Saitama City
Tel. +81 48 647 7217
japan@bmglabtech.com

UK
BMG LABTECH Ltd.
5 Alton House Office Park
Gatehouse Way
Aylesbury
HP19 8YB
Tel. +44 1296 336650
uksales@bmglabtech.com

USA
BMG LABTECH Inc.
13000 Weston Parkway
Suite 109
Cary, NC 27513
Tel. +1 877 264 5227
usa@bmglabtech.com

www.bmglabtech.com

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