The best microplate reader technology for neuro-degenerative disease research

- Creutzfeld-Jakob
- Dementia
- Scrapie
- Chronic wasting
- Alzheimer’s
- Parkinson’s
- BSE (Mad Cow)
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
- 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
- Brown et al., Sci Rep. 2016: β-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

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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. 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).

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.
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

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

**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 immunoassay, 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.

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.

**Conclusions**
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).

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
Detection modes
- Fluorescence intensity - incl. FRET
- AlphaScreen®/AlphaLISA®
- Luminescence (flash and glow) - incl. BRET
- Time-resolved fluorescence
- TR-FRET
- UV/Vis absorbance spectra

Measurement modes
- Top and bottom reading
- Endpoint and kinetic
- Sequential multi excitation
- Sequential multi emission
- Spectral scanning (absorbance)
- Ratiometric measurements
- Well scanning

Microplate formats
- 6 to 384-well plates, user-definable
- LVis Plate with 16 low volume microspots (2 µL)

Microplate carrier
- Robot compatible

Light sources
- High energy xenon flash lamp

Detectors
- Low-noise photomultiplier tube
- CCD spectrometer

Wavelength selection
- Optical filters: ex and em wheels for 8 filters each
- UV/Vis absorbance spectrometer: full spectra or 8 distinct wavelengths in < 1 sec/well

Optical filters
- Excitation and emission filter wheels for 8 filters each

Optical path guides
- Top: liquid filled light guides
- Bottom: fiber optics

Spectral range
- Filters: 240 - 740 nm or 240 - 900 nm for FI, FP, TRF
- 240 - 740 nm for LUM
- Spectrometer: 220 - 1000 nm for ABS

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
- AlphaScreen®: < 5 pM (< 100 amol/well P-Tyr-100, 384sv, 20 µL)*
- ABS with spectrometer: Full spectrum captured in < 1 s/well
  Selectable spectral resolution: 1, 2, 5, and 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 (1 flash): 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
- +4 °C above ambient up to 45 °C or 65 °C
  The upper heating plate of the incubation chamber operates at 0.5 °C more than the lower plate.
  This prevents condensation build-up on the lid or sealer.

Software
- Multi-user Reader Control and MARS Data Analysis Software included
- FDA 21 CFR Part 11 compliant

Dimensions
- Width: 44 cm, depth: 48 cm, height: 30 cm, weight: 28 kg

Optional accessories
- LVis Plate: Sample capacity: sixteen separate microdrop wells for 2 µL samples; one standard cuvette position for up to 1 mL samples.
  Quality control internal standards (optional): four NIST traceable optical density filters (approximate values of 0.1, 0.3, 0.6 and 1.0 OD); one holmium oxide filter for wavelength accuracy
  Dimensions: conforms to SBS standards for microplates.
- Atmospheric Control Unit: Actively regulates O₂ and CO₂ - 0.1-20%
- Stacker: Magazines for up to 50 plates - continuous loading feature
- THERMOstar: Microplate incubator and shaker
- Optical filters: Excitation and emission filters

Upgrades
- Please contact your local representative for upgrades including options such as detection modes, reagent injectors, etc.

* 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.

LOD = 3 x SD (20 blanks) / slope (6 pt std curve)
AlphaScreen® P-Tyr-100 assay kit, PerkinElmer, #6760620C
Microplates: white for LUM, AlphaScreen®, TRF; black for FI, FP; clear for ABS
96 = 96-well microplates
384sv = 384-well small volume microplates
384g = 384-well glass bottom microplates
**Detection modes**
- Fluorescence intensity - including FRET
- Fluorescence polarization/anisotropy
- AlphaScreen®, AlphaLISA®, AlphaPlex™
- Luminescence (flash and glow) - including BRET
- Time-Resolved Fluorescence - including TR-FRET
- UV/Vis absorbance

**Measurement modes**
- Top and bottom reading
- Endpoint and kinetic
- Sequential multi-excitation
- Sequential multi-emission
- Spectral scanning (fluorescence, luminescence, absorbance)
- Ratiometric measurements
- Well scanning

**Microplate formats**
- 6- to 1536-well plates, user-definable
- LVis Plate with 16 low volume microspots (2 µL)

**Microplate carrier**
- Robot compatible

**Light sources**
- High energy xenon flash lamp
- Dedicated laser for AlphaScreen®, AlphaLISA®, AlphaPlex™

**Detectors**
- Low-noise photomultiplier tube
- CCD spectrometer

**Wavelength selection**
- **Dual Linear Variable Filter (LVF) Monochromators™**
  - Linear Variable Dichroic Mirror: separates ex & em LVF Monochromators
  - Optical filters: Ex and em slides hold 4 filters each
  - LVF Monochromators + optical filters: Use one for ex and the other for em
  - UV/Vis absorbance spectrometer: Full spectrum or 8 distinct wavelengths in < 1 sec/well

**Optical filters**
- Excitation and emission slides for 4 filters each

**Optical path guides**
- Top and bottom: free air optical light path guided by motor-driven mirrors and dichroics

**Z-Adjustment**
- Automatic focal height adjustment (0.1 mm resolution)

**Spectral range**
- Filters: 240 - 750 nm or 240 - 900 nm for FL, FP, TRF
- LVF Monochromators™: 320 - 850 nm for FL, LUM
- Linear Variable Dichroic: 340 - 740 nm for FL, LUM
- Spectrometer: 220 - 1000 nm for Abs

**Sensitivity**
- FI Filters (top): < 0.15 µM fluorescein (< 3 amoL/well, 384wv, 20 µL)
- FI Filters (bottom): < 1.0 µM fluorescein (< 50 amoL/well, 384wv, 50 µL)
- FI Monochromator (top): < 0.35 µM fluorescein (< 7 amoL/well, 384wv, 20 µL)
- FI Monochromator (bottom): < 3.0 µM fluorescein (< 150 amoL/well, 384wv, 50 µL)
- FP: < 0.5 mP SD at 1 nM fluorescein (384wv, 20 µL)
- TRF: < 20 µM europium, 384, 80 µL
- HTRF® (black and white microplates): Reader Control Kit (Eu) after 18h (384wv, 20 µL)
  - Delta F > 880 % (High Calibrator)
  - Delta F > 30 % (Low Calibrator)
- LUM: < 0.4 mP ATP (< 8 amoL/well, 384wv, 20 µL)
- Dynamic Range: 9 decades
- AlphaScreen® with laser: < 5 µM 100 amoL/well P-Tyr-100, 384wv, 20 µL
- Abs with spectrometer: Selectable spectral resolution: 1, 2, 5, and 10 nm
  - OD range: 0 - 4 OD
  - Accuracy: < 1% at 2 OD
  - Precision: < 0.5% at 1 OD and < 0.8% at 2 OD

**Read times**
- Flying mode (1 flash): 8 s (96), 15 s (384), 28 s (1536)
- 10 flashes: 19 s (96), 57 s (384), 3 min 4 s (1536)

**Reagent injection**
- Up to 2 built-in reagent injectors
- Individual injection volumes for each well: 3 to 500 µL (optionally up to 2 mL)
- Variable injection speed up to 420 µL/s
- Reagent backflushing

**Shaking**
- Linear, orbital, and double-orbital with user-definable time and speed

**Incubation**
- +3 °C above ambient up to 45 °C or 65 °C
  - The upper heating plate of the incubation chamber operates at 0.5 °C more than the lower plate
  - This prevents condensation build-up on the lid or sealer

**Software**
- Integrated fluorophore library
- Multi-user reader Control and MARS data analysis software included
- FDA 21 CFR part 11 compliant

**Dimensions**
- Width: 45 cm, depth: 51 cm, height: 40 cm, weight: 32 kg

**Optional accessories**
- LVVis Plate: Sixteen separate microwells for 2 µL samples; standard cuvette position
- Quality control internal standards (optional)
- Atmospheric Control Unit: Actively regulates O₂ and CO₂ - 0.1-20%
- Stacker: Magazines for up to 50 plates - continuous loading feature
- THERMOstar: Microplate incubator and shaker
- Optical filters: Excitation and emission slides for 4 filters each

**Upgrades**
- Upgrades to include options such as additional detection modes, reagent injectors, etc. are available.
- Please contact your local representative for more information

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*Limit of detection (sensitivity) was calculated according to the IUPAC standard: 3x(SDblank) / slope for which BMG LABTECH has an exclusive license for the microplate reader market.*

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.

**Specifications are subject to change without notice.**