PHERAstar® *FSX*

The Gold Standard for High-Throughput Screening

BMG LABTECH

The Microplate Reader Company

"We found that the improvements in the new PHERAstar *FSX* outperformed our historic experiences and the other readers in our trial. The reader has fitted well into our assay development and screening groups, where it has been used frequently for a variety of technologies."

Mark Wigglesworth,
Director of High-Throughput Screening, AstraZeneca, Macclesfield, UK

The best performance in all assays

The PHERAstar® *FSX* was specifically conceived for the highest sensitivity and the fastest speed required in screening campaigns. Its unique features make it superior to any other microplate reader currently on the market. High-end German engineering combined with the latest technologies makes the PHERAstar *FSX* the gold standard microplate reader for High-Throughput Screening (HTS). This multi-mode plate reader performs all the leading detection technologies:

- UV/vis absorbance
- Fluorescence intensity, including FRET
- Luminescence (flash and glow), including BRET
- Fluorescence polarization/anisotropy
- Time-resolved fluorescence, including TR-FRET
- AlphaScreen®, AlphaLISA® and AlphaPlex™

Whatever your application, the PHERAstar *FSX* provides uncompromised sensitivity, speed and dynamic range in all plate formats up to 3456 wells.

Three dedicated light sources, Simultaneous Dual Emission, Decay Curve Monitoring and assay-dedicated Optic Modules are just some of its key features. Assay flexibility is enhanced by software-controlled top/bottom reading, on-board reagent injectors, precise temperature control and multi-mode shaking capabilities.

Any plate format.

Sensitivity and speed

The PHERAstar *FSX* combines fast read times necessary for HTS with the sensitivity to read small volumes. The user can always find the best combination of sensitivity and speed by choosing the number of excitation flashes. In single flash mode, the reader can measure a 1536-well plate in 27 seconds - one of the fastest readers on the market. Even at low concentrations and small assay volumes, the unsurpassed sensitivity of its detection system provides outstanding S/N, %CV, and Z' values.

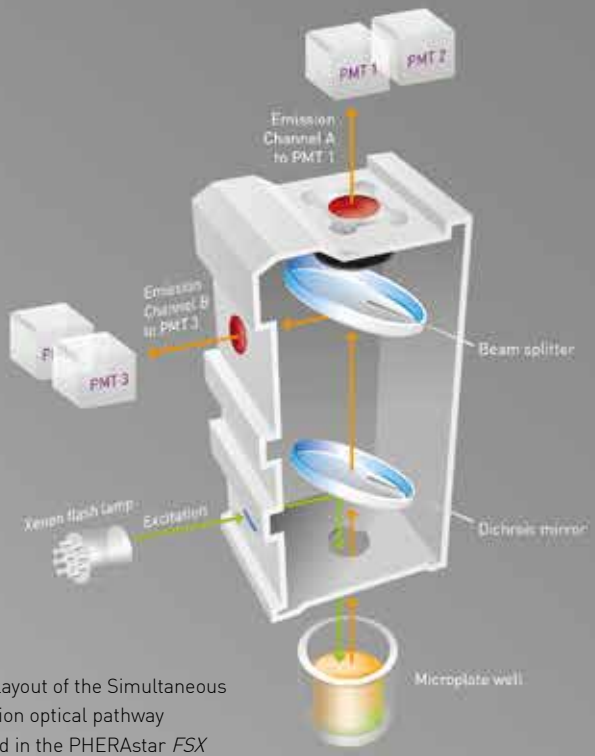
Innovative technology

The outstanding sensitivity of the PHERAstar *FSX* is centered on an innovative lens-based optical design composed of a free air optical path, three independent light sources, Simultaneous Dual Emission detection, and high transmission filters. Depending on the application, users can choose one of the following light sources:

- High energy xenon flash lamp
- Laser for TRF / TR-FRET
- Laser for AlphaScreen®, AlphaLISA® and AlphaPlex™

Assay-specific Optic Modules, fully-equipped, installed in seconds.

Four photomultiplier tubes (PMTs), combined in two application-dedicated matched pairs, are used as detectors. One pair is dedicated to simultaneous dual fluorescence and luminescence detection, the second to TRF-based signals.



Schematic layout of the Simultaneous Dual Emission optical pathway incorporated in the PHERAstar *FSX*

Switch assays in seconds

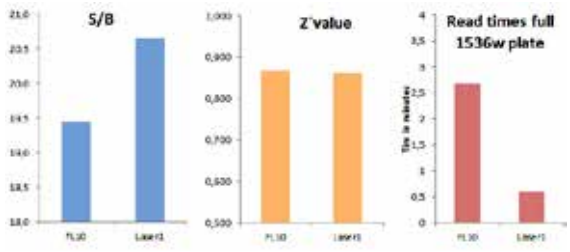
Never worry about which filters or dichroic mirrors are installed. Our application-specific Optic Modules make the PHERAstar *FSX* the easiest reader to optically configure on the market. Optic Modules contain all the components required for a specific assay such as excitation and emission filters, dichroic mirrors, beam splitters and polarization filters. The modules are automatically recognized via barcode and selected by the reader for the appropriate assay. The reader accommodates up to six Optic Modules. You can easily add or replace them within seconds.

Cut read times in half

Numerous assays require detection of two emission wavelengths. BMG LABTECH pioneered the technique of Simultaneous Dual Emission (SDE) detection for microplate readers. Thanks to SDE detection, the PHERAstar *FSX* can simultaneously detect two separate emission wavelengths in one single measurement. SDE detection can be used in any assay that measures two emission wavelengths or polarization vectors, including FRET, TR-FRET, BRET, FP and AlphaPlex™. It offers a significant speed advantage by cutting read times in half and eliminates the typical drawbacks of double sequential detection such as flash-to-flash variations, photobleaching, decaying kinetic signals. SDE also reduces the variability caused by fluctuations in pH or temperature, and by evaporation.

TRF-dedicated excitation laser

Dedicated lasers for excitation significantly improve assay performance and lower limits of detection as they yield a higher excitation energy at a specific wavelength.
The PHERAstar *FSX's* TRF laser specifically excites samples at 337 nm. With 60 laser flashes per second, it allows for ultra-fast TR-FRET/HTRF® measurements and even "flying mode" detection. For several applications, a single laser flash in flying mode provides enough energy to excite donor molecules. Measurements can therefore take place without stopping plate movement, significantly reducing read times for an entire plate.



Comparison of xenon lamp (10 flashes; FL10) and TRF laser (1 flash; Laser1) for Signal-to-Blank (S/B), Z' value and read times with a HTRF assay.

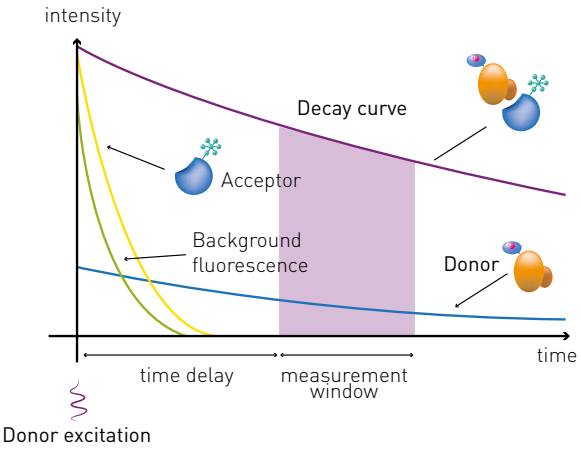
Advanced Alpha Technology detection

The new combination of laser excitation and Simultaneous Dual Emission detection gives the PHERAstar *FSX* unsurpassed flexibility and sensitivity for all AlphaScreen®, AlphaLISA® and AlphaPlex™ assays. The dedicated laser for Alpha Technology specifically excites donor beads at 680 nm and provides a broad dynamic range and an increased signal-to-noise ratio. In addition, the outstanding SDE detection system allows for reduced read times and higher sensitivities in all multiplex Alpha assays.

Top/bottom focal height adjustment

Thanks to the innovative free air optic path system which uses a series of software-controlled mirrors to directly focus the light beam on the bottom or top of the plate, light transmission is significantly enhanced. This results in unmatched top and bottom reading performance. Top or bottom detection is automatically adjusted by the software, requiring no hardware changes.

Automated focal height adjustment at a resolution of 0.1 mm can be performed for both top and bottom readings. This feature ensures the best signal-to-noise ratio for every application, eliminating the influence of microplate formats, sample volumes, surface tension and evaporation in all plate formats up to 3456.



Unique TRF Decay Curve Monitoring feature for assay optimization.

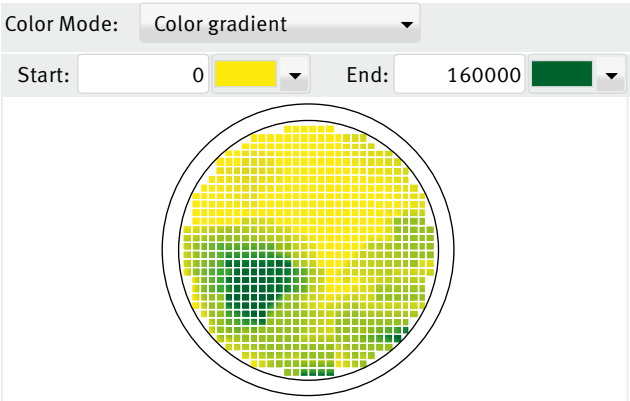
Unique Decay Curve Monitoring

Decay Curve Monitoring (DCM) is a unique feature of the PHERAstar *FSX* and a fundamental tool for assay development and performance fine-tuning in TRF, TR-FRET and Alpha Technology. Enabled by a dedicated photon counting detection system, DCM measures and visualizes the time-resolved emission curve of the fluorophore. The TR-FRET dedicated photon counting detection system enables simultaneous monitoring of both donor and acceptor decay curves with a time resolution of 2 µs. Combined with the Integration Time Wizard, DCM helps to optimize timing parameters, thus improving signal detection and reducing background noise.

Advanced Well Scanning

Conventional readers excite and measure the emission light of the samples in the center of the well. This can affect the reliability of measurements of uneven cell layers or non-homogenous well samples.
Thanks to the Matrix Scan mode, the PHERAstar *FSX* can scan the whole well surface, taking multiple measurements with a resolution of up to 900 data points/well. The software

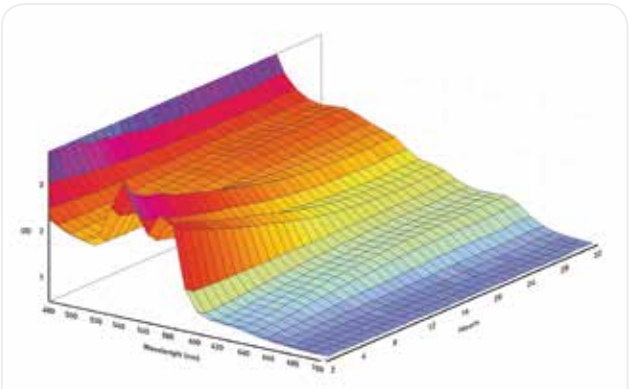
displays each scan point graphically and creates a map for each well. Alternatively, orbital or spiral averaging can be applied to measure heterogeneous well content. In these modes, the reader takes several measurements on a defined orbit and calculates an average for each well.



Matrix Scanning of GFP-expressing protoplasts. The scan shows how protoplasts (green colour) are not evenly distributed in the well.

Ultra-fast detection of absorbance spectra

BMG LABTECH was the first company to introduce an absorbance spectrometer into a multi-mode HTS plate reader. This technology can capture a full UV/vis absorbance spectrum (220 - 1000 nm) in less than one second/well, significantly faster than any absorbance monochromator. Absorbance spectra can be detected at resolutions selectable from 1 to 10 nm. Alternatively, you can simultaneously measure up to 8 discrete wavelengths in a single measurement in less than one second per well.



Time-dependent change in haemoglobin absorbance spectrum in the presence of *N.nigricollis* venom.

Smart reagent injection

Many assays require the ability to monitor a signal before, during and after the addition of a reagent to a well. The two built-in reagent injectors on the PHERAstar *FSX*, allow for simultaneous detection and reagent injection. This ensures that even fast kinetic reactions are monitored without the loss of any data point. Injectors are readily accessible and housed within the instrument to protect any light-sensitive reagent.

Exceptionally small dead volume and back flushing significantly reduce wastage of expensive reagent. The number of injections/well and injection volumes are adjustable for each well, allowing you to automatically produce dilution schemes and concentration gradients across the microplate.

Automation

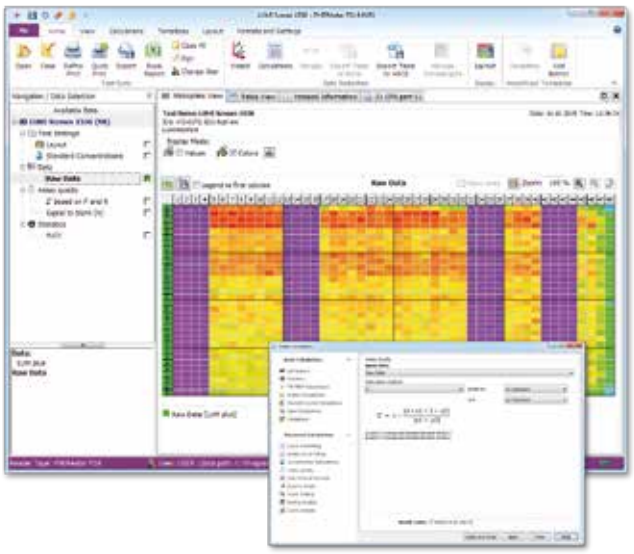
For HTS automation purposes, the PHERAstar *FSX* offers excellent robotic integration capabilities, multi-user control and MARS data analysis software with digital signature and FDA 21 CFR part 11 compliance. Its standardized small reader footprint and robotic software interface make it easy to integrate into all leading robotic platforms. The instrument comes with three integrated microplate barcode readers capable of reading the east, west and south side of a microplate and can be equipped with BMG LABTECH's Stacker.

Microplate Stacker

The Stacker is an ideal solution for mid-throughput labs that wish to have the small footprint of an automated plate feeder along with the simplicity and reliability the Stacker offers. It provides loading, unloading, restacking and a continuous load feature of up to 50 microplates. BMG LABTECH Control Software and the script mode give the user unlimited flexibility to run diverse assays.

Control and MARS data analysis software

The PHERAstar *FSX* software package provides an extensive range of possibilities for both test protocol definitions and data analysis and is fully compliant with FDA regulation 21 CFR Part 11. The Control Software allows users to define instrument parameters and test protocols.



MARS Data Analysis Software for automated data reduction.

The MARS data analysis software allows the user to display and process data with only one mouse click using predefined templates.
The software is also capable of creating standard curves based on the following curve fitting algorithms to calculate values such as EC_{50}, IC_{50} and r^2:

- Linear regression fit
- 4 and 5 parameter fit
- Segmental regression fit
- Cubic spline fit
- 2nd and 3rd polynomial fit
- Point-to-point fit
- Hyperbola fit
- User defined fit
- Enzyme kinetic (e.g. Michaelis-Menten; Lineweaver-Burk)

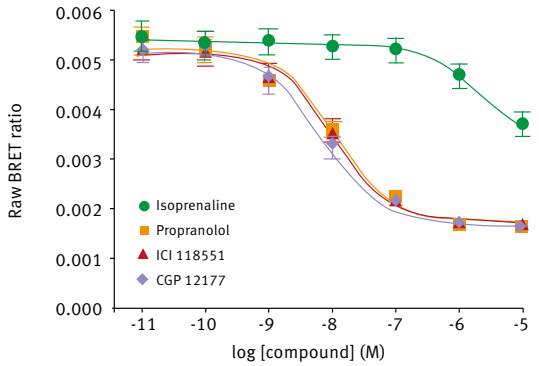
The MARS Standard Curve Wizard creates a step-by-step calculation of a standard curve and important parameters such as S/N, Delta F % and Z' are easily obtained.

Applications center

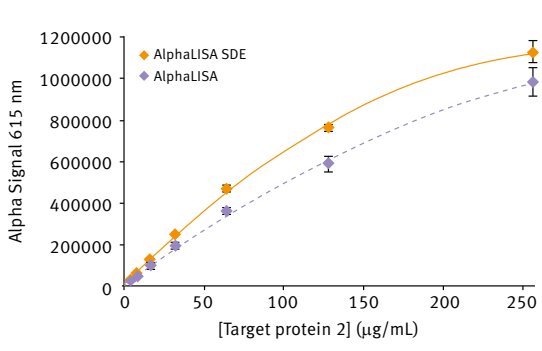
A perfectly engineered microplate reader is only part of the solution. The reader's ability to effectively perform all of the leading applications is the final piece of the puzzle. With the PHERAstar *FSX*, we offer a unique combination of features to support all major existing applications as well as future needs. Applications include:

- Protein-protein interactions
- Kinetic binding assays
- Compound and inhibitor screening
- DNA, RNA, and protein quantification
- Enzyme activity and kinetic assays
- Cell based assays
- Reporter gene assays

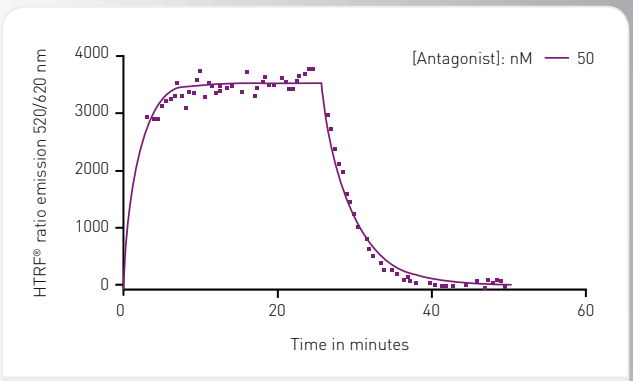
The reader provides excellent performance in all HTS applications, including NanoBRET™, AlphaScreen® and HTRF® assays, illustrated by the following examples:



NanoBRET competitive binding experiments of propranolol-BY630 with increasing concentrations of known unlabelled ß2AR ligands.



Comparison of AlphaLISA and AlphaLISA SDE module performance.



HTRF-based antagonist kinetic binding assay

We continuously work with all the leading reagent companies to optimize instrument settings for their existing assays and their newest chemistries.

Visit our Applications Center online to find references to all applications, listed as application notes, application focus, and peer-reviewed papers.

The comprehensive searchable applications database reflects more than 25 years of expertise and innovations in microplate reading technology. Over 4,000 references exemplify the flexibility and versatility of our readers, as well as their use in the chemical and biological sciences.

Support and training

BMG LABTECH operates globally through an extensive network of subsidiaries and trained distributors. Customers can rely on qualified support and assistance with regard to software, assay development, or general enquiries related to the PHERAstar *FSX* and all our other microplate reading solutions.

AlphaPlex™ technology
DNA/RNA quantifications
HTRF®
Transcreener®
Apoptosis
AlphaTechnology
LanthaScreen®
Binding studies
HTS
SNP Genotyping
ORAC
Ca^{2+} assays
Kinase activity
Enzyme activity
Gene expression
Protein quantifications
Dual luciferase assays
Reporter gene assays
BRET assays
Enzyme kinetics
ROS detection
Cell Viability
PCR product quantifications
NADH / NADPH assays
DELFIA®
LANCE®
Solubility tests
ATP and ADP detection
Immunoprecipitation
Protease activity
FRET assays
ELISA
DLR™

PHERAstar *FSX* - 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	UV/vis absorbance spectra Fluorescence intensity - incl. FRET Luminescence (flash and glow) - incl. BRET Fluorescence polarization Time-resolved fluorescence TR-FRET AlphaScreen®/AlphaLISA®/AlphaPlex™	
Measurement modes	Top and bottom reading Endpoint and kinetic Sequential multi-excitation Sequential multi-emission Simultaneous Dual Emission Spectral scanning (absorbance) Real-time ratiometric measurements Well scanning	
Microplate formats	Up to 3456-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™ Dedicated laser for TRF and TR-FRET	
Detectors	Two matched pairs of photomultiplier tubes (PMTs), optimized for different detection modes CCD spectrometer	
Wavelength selection	Optic Modules: up to six application-specific modules (includes all filters, dichroics, etc., for an application) For top and bottom reading UV/vis absorbance spectrometer: full spectrum or up to 8 distinct wavelengths in < 1 sec / well	
Optical filters	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**	230 - 750 nm or 230 - 900 nm for FI, FP 230 - 750 nm for LUM 230 - 900 nm for TRF
	Spectrometer	220 - 1000 nm for ABS
Sensitivity	**FI** (top)	< 0.15 pM fluorescein (384sv black, 20 µL) < 0.5 pM fluorescein (1536 black, 8 µL)
	FI (bottom)	< 1.0 pM fluorescein (384g black, 50 µL)
	FP	< 0.5 mP SD at 1 nM fluorescein (384sv black, 20 µL) < 1.5 mP SD at 1 nM fluorescein (1536 black, 8 µL)
	TRF with laser	< 5 fM europium (384, 80 µL) < 15 fM europium (1536, 8 µL)
	HTRF® (black and white microplates)	Reader Control Kit (Eu) after 18h (384sv, 20 µL) Delta F > 1100 % (High Calibrator) Delta F > 25 % (Low Calibrator)
	LUM	< 0.4 pM (< 8 amol/well ATP, 384sv white, 20 µL) Dynamic range: 9 decades
	AlphaScreen® with laser	< 5 pM (< 100 amol/well P-Tyr-100, 384sv white, 20 µL)*
	ABS with spectrometer	Full spectrum captured in < 1 s / well 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)	14 s (384) 27 s (1536)
	10 flashes	38 s (384) 1 min 52 s (1536)
	50 flashes	1 min 29 s (384) 5 min 18 s (1536)
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, optionally up to 2 mL) 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	
Integrated barcode reader	Up to three integrated barcode readers	
Incubation	+5 °C above ambient up to 45 °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: 45 cm, depth: 51 cm, height: 47 cm; weight: 49 kg	
	Optional 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. Incorporates NIST-traceable filters and holmium oxide standards for instrument performance test. Sensitivity: 2 ng/µL dsDNA	
Upgrades	Please contact your local representative for upgrades including options such as detection modes, reagent injectors, etc.	

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

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

Specifications are subject to change without notice.

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