PHERAstar® *FSX*

The new Gold Standard for High-Throughput Screening

BMG LABTECH

The Microplate Reader Company

The new reference microplate reader for HTS

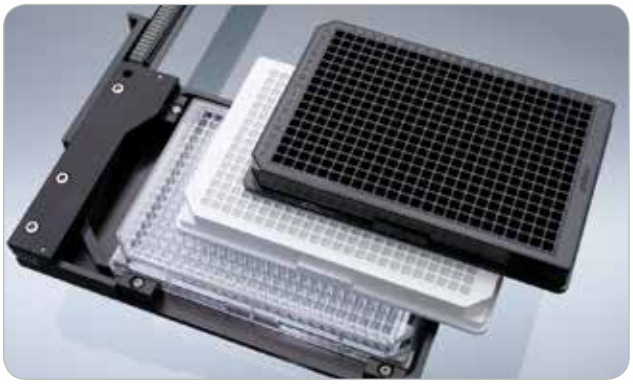
Whether you need speed, sensitivity or flexibility, the PHERAstar *FSX* is the ultimate microplate reader for your research and HTS applications.

The best performance in all assays

Built upon the successful PHERAstar *FS* platform, the PHERAstar® *FSX* was specifically conceived for the highest sensitivity and the fastest speed. Its new and unique features make the PHERAstar *FSX* superior to any other microplate reader currently on the market. High-end German engineering combined with the latest technologies makes the PHERAstar *FSX* the new Gold Standard microplate reader for HTS. The PHERAstar *FSX* multi-mode plate reader performs all the leading detection technologies:

- Ultra-fast UV/Vis Absorbance Spectra
- Fluorescence Intensity, including FRET
- Fluorescence Polarization/Anisotropy
- Time-Resolved Fluorescence, including TR-FRET
- High-end Alpha Technology, including AlphaPlex™
- Luminescence (flash and glow), including BRET

The PHERAstar *FSX* provides uncompromised sensitivity, speed and dynamic range in all plate formats up to 3456. Sequential Dual Excitation, Simultaneous Dual Emission and ratiometric calculations are just some of the key features of the PHERAstar *FSX* for multichromatic applications such as FRET, TR-FRET, BRET and FP. Assay flexibility is enhanced by software-controlled top/bottom reading, onboard reagent injectors, precise temperature control and multi-mode shaking capabilities.



Any plate format.

Innovative optical design

The outstanding sensitivity of the PHERAstar *FSX* is based on a new, innovative lens-based optical design which is

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™

As detectors, the PHERAstar *FSX* uses two matched pairs of photomultiplier tubes (PMTs). The first PMT pair is dedicated to simultaneous luminescence and fluorescence detection, whereas the second pair detects time-resolved-fluorescence based signals.

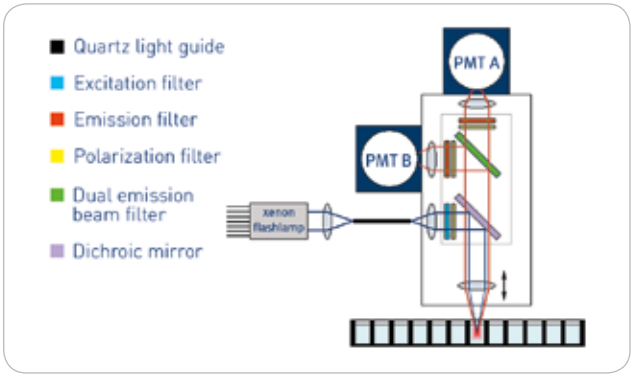
Assay-specific Optic Modules

Never worry about which filters or dichroic mirrors are installed in your PHERAstar *FSX*. BMG LABTECH's application-specific Optic Modules are easy to handle and contain all the optical components required by a specific assay such as excitation and emission filters, dichroic mirrors, beam splitters and polarization filters. Optic Modules are barcoded and are automatically recognized and selected by the reader for the appropriate assay. Up to six Optic Modules can be accommodated in the PHERAstar *FSX*. Users can easily add or replace modules within seconds.

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

Simultaneous Dual Emission

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. This offers a significant speed advantage by cutting read times in half. SDE corrects flash-to-flash variations, photobleaching, decaying kinetic signals, or fluctuating conditions like temperature, pH, and evaporation. SDE detection can be used in any assay that measures two emission wavelengths or polarization vectors, including FP, FRET, HTRF® and AlphaPlex™.



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

TRF-dedicated excitation laser

Dedicated lasers for excitation significantly improve assay performance and lower limits of detection. 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.

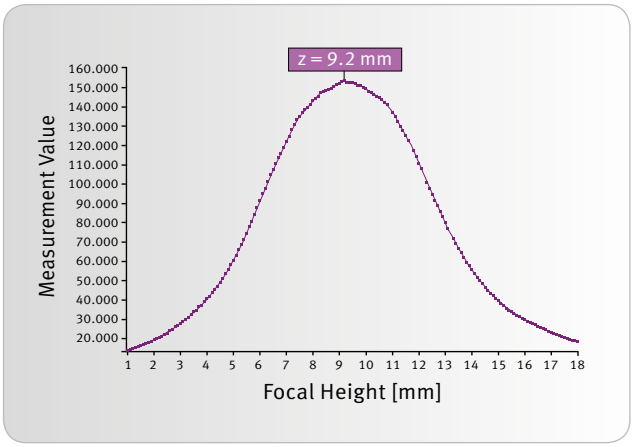
Advanced Alpha Technology detection

The new combination of laser excitation and Simulataneous 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.

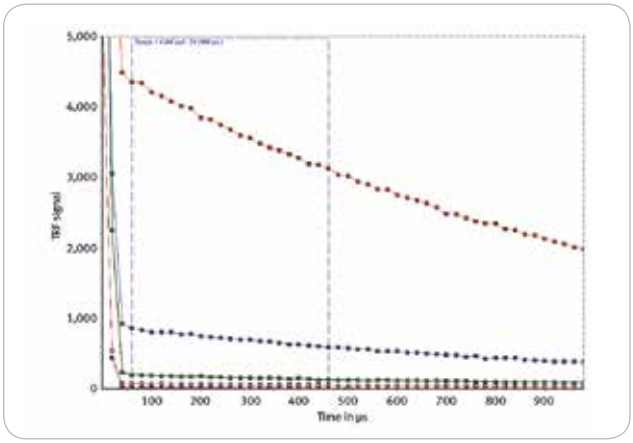


The automated focal height adjustment ensures the best signal-to-noise ratio in all detection modes and all plate formats.

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.

Decay Curve Monitoring

A fundamental tool for assay development and optimization in TRF, TR-FRET and AlphaTechnology, Decay Curve Monitoring (DCM), is a unique feature of the PHERAstar *FSX*. DCM measures and visualizes the fluorophore's time-resolved emission curve and helps to optimize timing parameters, thus improving signal detection and reducing

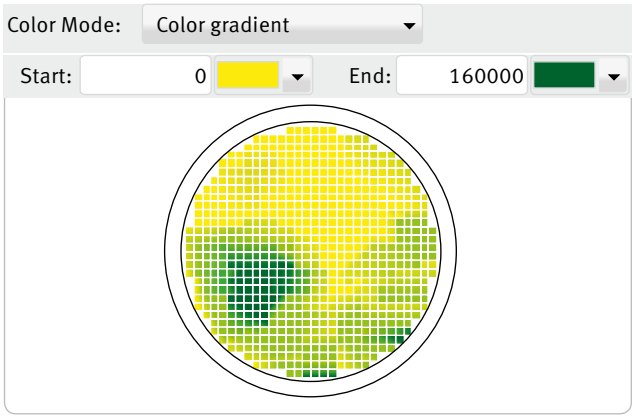


Unique TRF Decay Curve Monitoring feature for assay optimization.

background noise. The dedicated photon counting detection system for TR-FRET allows simultaneous monitoring of the donor and acceptor decay curves with a time resolution of 2 µs.

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 in uneven cell layers or nonhomogenous well samples. Thanks to the Well Scanning mode, the PHERAstar *FSX* scans the whole well surface and takes 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, BMG LABTECH's unique Orbital Averaging can be used to measure heterogeneous well content. In this

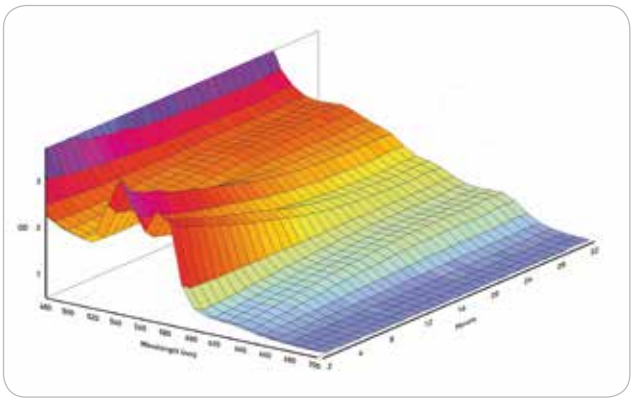


Well Scanning of GFP-expressing protoplasts. The well scan shows that the protoplasts (green colour) are not evenly distributed in the well.

mode, the reader takes several measurements on a defined orbit and calculates an average for each well.

Ultra-fast absorbance spectrometer

BMG LABTECH was the first company to integrate a spectrometer for absorbance measurements into a multi-mode HTS microplate reader. This technology can capture a full UV / Vis absorbance spectrum (220 - 1000 nm) in less than one second per well, significantly faster than any absorbance monochromator. An absorbance spectrum can be detected at resolutions selectable from 1 to 10 nm. Alternatively, users can simultaneously measure up to eight 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 popular assays require the ability to monitor a signal before, during and after the addition of a reagent to a well. In the PHERAstar *FSX*, the two built-in reagent injectors are ideally positioned so reagents can be added to the well currently being read. This ensures that even fast kinetic reactions can be monitored without the loss of any data point. Injectors are readily accessible and housed within the instrument to protect any light-sensitive reagent. An exceptionally small dead volume and back flushing significantly reduce expensive reagent wastage. The number of injections/well and injection volumes are adjustable for each well, allowing users to automatically produce dilution schemes and concentration gradients across the microplate.

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 flashes. In single flash mode, the PHERAstar *FSX* can read a 1536-well plate in 27 seconds, making the PHERAstar *FSX* among the fastest in the microplate reader industry. Even at low concentrations and small assay volumes, the unsurpassed sensitivity of the PHERAstar *FSX* detection system provides outstanding S/N, %CV, and Z' values.

Automation

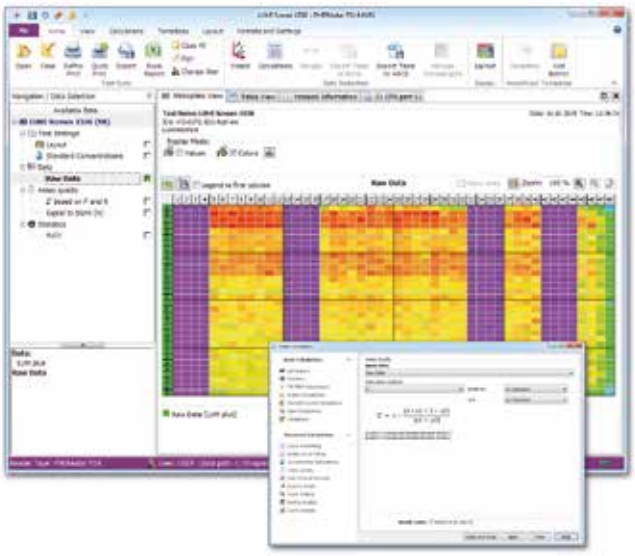
BMG LABTECH understands that automation is a key factor in HTS and has designed its microplate readers to be easily automated. All BMG LABTECH microplate readers have similar x-y dimensions and plate in/out locations. This minimizes the cost of automation solutions for customers. The many robotic integrations available are the result of BMG LABTECH's extensive collaboration with various robotic companies. The small standardized reader footprint and robotic software interface make it easy to integrate the PHERAstar *FSX* into all leading robotic platforms. Moreover, the PHERAstar *FSX* comes with three integrated microplate barcode readers capable of reading the east, west and south side of a microplate.

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)

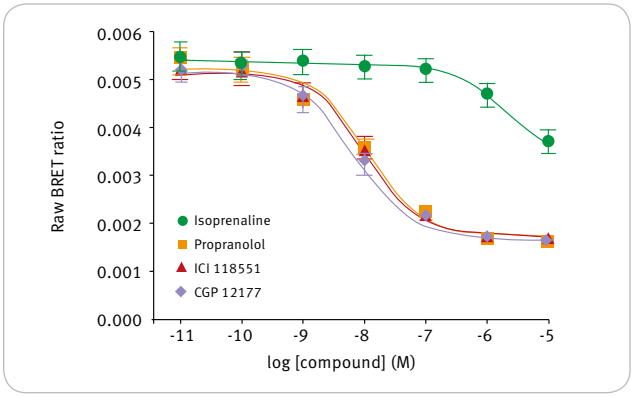
The MARS Standard Curve Wizard creates a step-by-step calculation of a standard curve and important parameters such as S/N, Delta F % and Z' are easily obtained.

Applications center

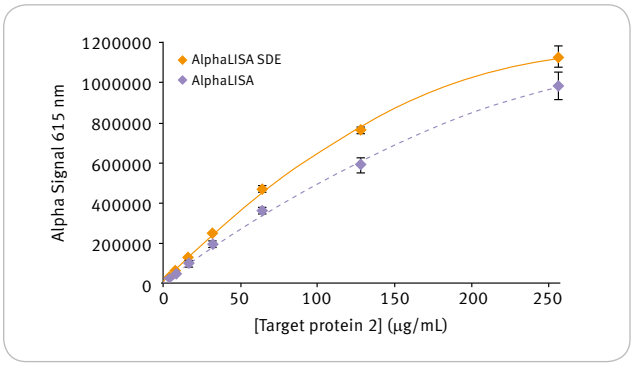
A perfectly engineered microplate reader is only part of the solution. The reader's ability to effectively perform all of the leading applications is the final piece of the puzzle. With the PHERAstar *FSX*, BMG LABTECH offers a unique combination of features to support all major existing applications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

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

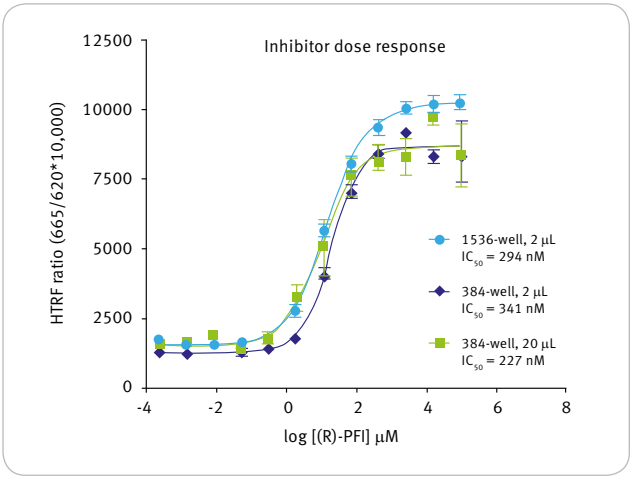
The PHERAstar *FSX* provides excellent performance in all HTS applications, including NanoBRET, AlphaScreen® and HTRF® assays, illustrated by the following examples:



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



Comparison of AlphaLISA and AlphaLISA SDE module performance.



(R)-PFI 2 hydrochloride inhibitor titration with SET7/9 enzyme detected with HTRF.

BMG LABTECH continuously works with all the leading reagent companies to optimize instrument settings for their existing assays and their newest chemistries.

Visit BMG LABTECH's 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 other BMG LABTECH 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	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 Spectra	
Measurement modes	Top and bottom reading Endpoint and kinetic measurements Simultaneous Dual Emission measurements Sequential multi excitation measurements Sequential multi emission measurements Real-time ratiometric measurements Well scanning	
Microplate formats	Up to 3456-well plates, user-definable	
Light sources	High energy xenon flash lamp Laser for TRF and TR-FRET Laser for AlphaScreen®, AlphaLISA®, AlphaPlex™	
Detectors	Two matched pairs of photomultiplier tubes, optimized for different detection modes	
Optic Module capacity	Up to six application-specific and barcoded Optic Modules built in	
Z-adjustment	Top/bottom automatic focal height adjustment (0.1 mm resolution)	
Spectral range	230 - 750 nm or 230 - 900 nm for FI, FP 230 - 750 nm for LUM 230 - 900 nm for TRF 220 - 1000 nm for ABS	
Sensitivity	FI (top)	< 0.15 pM fluorescein (black 384sv, 20 µL) < 0.5 pM fluorescein (black 1536, 8 µL)
	FI (bottom)	< 1.0 pM fluorescein (black 384 glass bottom, 50 µL)
	FP	< 0.5 mP SD at 1 nM fluorescein (black 384sv, 20 µL) < 1.5 mP SD at 1 nM fluorescein (black 1536, 8 µL)
	TRF	< 5 fM europium (white 384, 80 µL) < 15 fM europium (white 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 ATP (< 8 amol/well, white 384sv, 20 µL) Dynamic range: 9 decades
	AlphaScreen®	< 5 pM (< 100 amol/well P-Tyr-100, white 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 - 4 OD Accuracy: < 1% at 2 OD Precision: < 0.5% at 1 OD and < 0.8% at 2 OD
Read times	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 onboard reagent injectors Injection at measurement position (6 to 384-well) Injection volumes for each well 3 to 500 µL (optional 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	
Barcode reader	Up to three integrated microplate barcode readers	
Incubation	+5 °C above ambient to 45 °C	
Software	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: 47 cm; weight: 49 kg	
	Accessories	
Stacker	Magazines for up to 50 plates - continuous loading feature	
THERMOstar	Microplate Incubator and Shaker	
Optic Modules	Available for all applications	
Upgrades	Upgrades to include options such as additional detection modes, reagent injectors, 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

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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