

NEPHELOstar *Plus*

Laser-based **Microplate Nephelometer**
that Measures Light Scattering





The unique laser-based microplate
Nephelometer
that measures scattered light.

The NEPHELOstar *Plus* is the world's only laser-based microplate nephelometer that uses light scattering to measure turbidity or solubility of a sample.

Flexibility

The NEPHELOstar *Plus* microplate nephelometer detects particles in liquid samples by measuring forward scattered light when a laser beam is directed through the solution. This scattered light is detected at incident angles up to 80 degrees, making it approximately thirty times more sensitive than traditional transmission readers that measure the reduction in direct light passing through a sample well.

The key feature of the NEPHELOstar *Plus* is the robust optical system employing a self-monitoring laser diode that offers adjustable intensity and beam diameter. These features permit the user to reduce meniscus effects and to optimize sensitivity, allowing for measurements to be performed in up to 384-well plate formats.



Laser light is scattered in a microplate well due to insoluble particles in the solution.

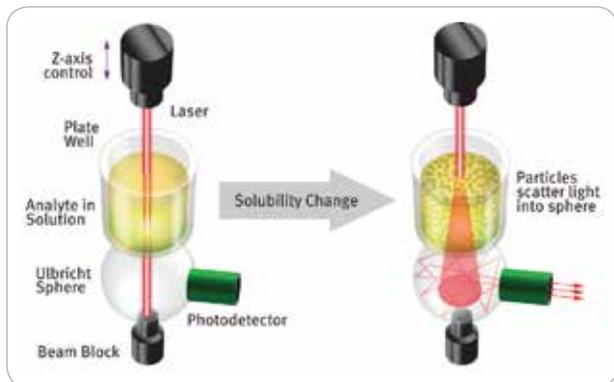
Up to two onboard reagent injectors, precise temperature control, multi-mode shaking capabilities, automatic gain adjustment, Stacker plate handler, and compatibility with robotic systems further enhance instrument flexibility.

Optical Design

The high-intensity light source of the NEPHELOstar *Plus* is a laser diode (at 635 nm) with a highly collimated beam. The laser beam passes through the sample well into an Ulbricht sphere scattered light detector. If the light is not deflected by particles, it passes straight through the sphere and no signal is generated. If particles are present in the sample, the light is scattered and reflected around the interior of the sphere and ultimately detected by a photodiode that records a signal.

In liquid solutions, the relationship between the concentration of scattering particles and scattered light intensity is linear

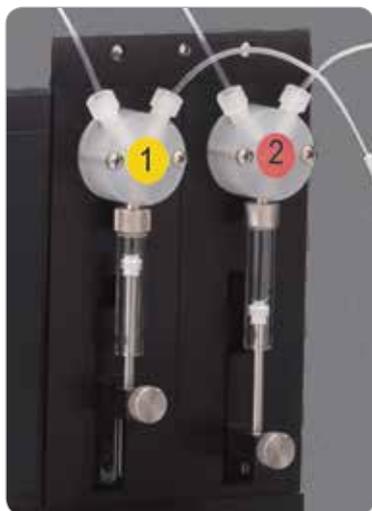
over a wide range of concentrations. An exclusive feature of the NEPHELOstar *Plus* is the ability to adjust the laser intensity and the beam width for best performance. With a narrow beam width, liquid surface effects such as a strong meniscus are reduced.



*Nephelometry: Scattered light is detected at incident angles of up to 80 degrees, making the NEPHELOstar *Plus* more sensitive than traditional transmission readers.*

Liquid Handling

Two precise onboard injectors allow simultaneous reagent injection and detection. Thus, concurrent reading and injection ensures that even the initial part of fast kinetic reactions can be monitored. Kinetic data can be collected as fast as 50 reading points per second or as slow as one measurement every 2.5 h. The injectors are readily accessible and are housed within the instrument to safeguard any light sensitive reagents.



...up to two reagent injectors, programmable with individual injection volumes for each well

The exceptionally small dead volume and back flushing ensure that precious reagents are conserved. Users can tune all parameters, such as plate shaking, injection speed, timing, and the number of injections per well to automatically produce dilution schemes and concentration gradients across the microplate.

Assays

The flexibility and performance of the NEPHELOstar *Plus* allows more applications to be adapted to microplate-based laser nephelometry than ever before.

Flocculation assays, drug solubility determination, bacterial and fungal growth kinetics, and determination of precipitation of particles in solution are amongst a variety of possible studies. Four examples are outlined below:

- **Automated Drug Solubility Screening**
Determining aqueous compound solubility has become an essential early measurement in the drug discovery process to avoid time-consuming and costly ADME screens of low solubility compounds. Developed to meet high-throughput demands, the NEPHELOstar *Plus* offers HTS/drug screening laboratories a fast and simple method for checking compound solubility, which can be fully automated. The nephelometric method has been shown to produce results equivalent to those produced by an HPLC method and to be largely unaffected by colored solutions.
- **Microbial Growth Kinetics**
Continuous nephelometric monitoring of changes in the turbidity can be used to test antimicrobial drugs and their effects on microbial growth kinetics. Among various parameters of the growth curves, the duration of the lag phase is strongly affected by the presence of antimicrobial drugs. Using the NEPHELOstar *Plus* instead of a traditional photometer, this early part of the growth curve can be monitored more exactly. Featuring additional precise temperature control, multi-mode shaking capabilities, and a gas vent, the instrument is a perfect tool to study microbial growth.
- **Quantification of Proteins**
In clinical chemistry immunonephelometric assays are used to determine the concentration of serum immunoglobulin (IgA, IgG, IgM), complement

components (C3, C4), acute phase reactant proteins (CRP, transferrin), albumin, and α -1-antitrypsin. Protein precipitation of globular proteins refers to the formation of protein aggregates by adding e.g. salt or organic solvent. In contrast, immunoprecipitation allows a given protein to be precipitated selectively via an antibody-antigen reaction.

□ Monitoring of Polymerization

In organic chemistry, nephelometry is used to quantify macromolecules, e.g. by monitoring of a polymerization reaction.

The NEPHELOstar *Plus*' unique combination of features is ideal for all four application areas.

ACU Gas Controller Regulates CO₂ and O₂

An accessory to the NEPHELOstar *Plus* is the Atmospheric Control Unit (ACU). This microprocessor controlled regulator optimizes the levels of atmospheric gases, CO₂ and O₂, within the microplate reader chamber. This accessory is perfect for cell growth assays where CO₂ and O₂ need to be properly regulated. In addition to optimizing cellular growth conditions, biological conditions such as hypoxia or anoxia can be induced to see what effect there is on cell growth.



ACU gas controller regulates both CO₂ and O₂ within the NEPHELOstar *Plus* microplate chamber.

Stacker and Robot Compatibility

For medium level throughput, BMG LABTECH offers a Stacker that can be used with the NEPHELOstar *Plus*. 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.



The script mode of the Stacker software gives the user unlimited flexibility to run diverse assays. This function can be used to choose different test definitions for different plates in one batch run or to perform more than one measurement on one plate.

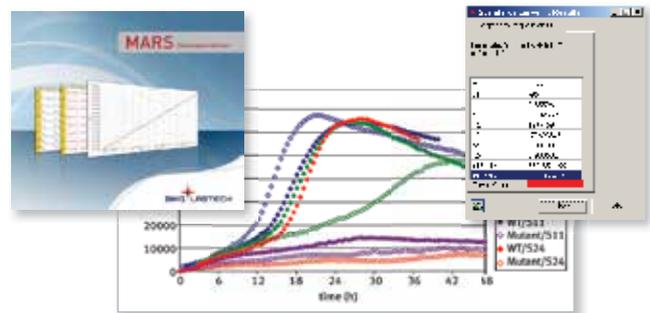
Automated microplate handling with Stacker.

Control and MARS Data Analysis Software

The NEPHELOstar *Plus* software package provides an extensive range of possibilities for both test protocol definitions and data analysis. The Reader Control part of the software allows users to define instrument parameters and test protocols, and MARS offers various tools to easily determine compound solubility or microbial growth kinetics.

Well organized, versatile, easy to use and powerful are just a few of the ways the MARS Data Analysis Software package is described by users. MARS provides several options to display data in a clear and concise format.

Data can be processed with powerful predefined templates or by using an extensive range of data calculation features. For example the automatic determination of compound solubility by a segmental regression fit can be done, or a standard curve can be generated based on different curve fitting algorithms to calculate EC₅₀, IC₅₀, and r² values.



The MARS Data Analysis Software provides sophisticated tools for automated data reduction.

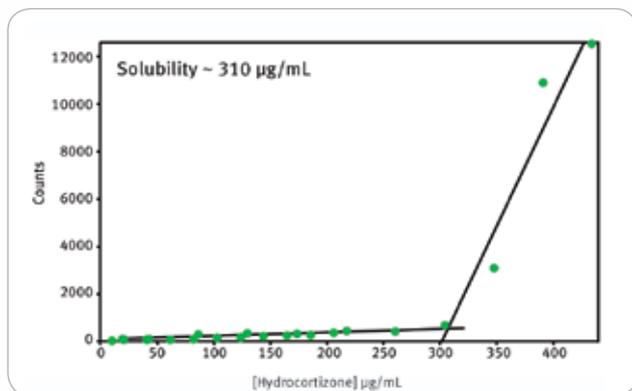
Applications Center

The NEPHELOstar *Plus* has been cited in numerous publications such as application notes, scientific posters, and peer-reviewed papers exemplifying the versatility of the NEPHELOstar *Plus*. The following main categories are amongst a wide range of possible applications:

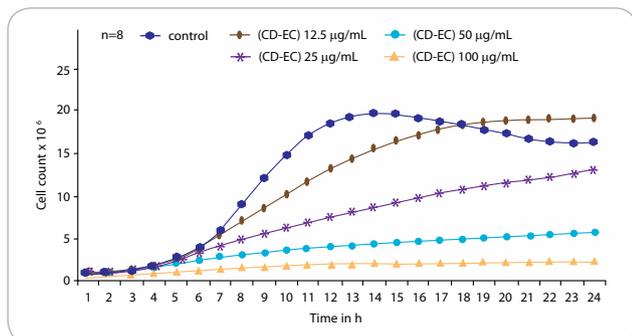
- Automated Drug Solubility Screening
- Microbial Growth Kinetics
- Quantification of Proteins

The NEPHELOstar *Plus*' versatility and flexibility are illustrated by the following examples for:

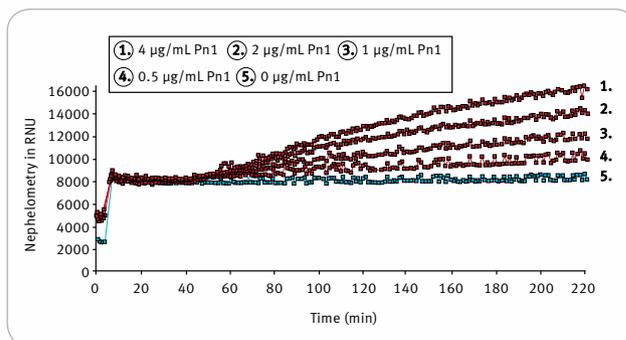
- Solubility screen of the corticosteroid hydrocortizone
- Monitoring of antifungal agents
- Antigen-antibody binding



Solubility screen of hydrocortizone. The intersection at 310 µg/L indicates the point of precipitation.¹



*The antifungal econazole nitrate complex (CD-EC) inhibits growth of *Candida albicans*.²*



Kinetic determination of antigen-antibody binding at different concentrations.³

BMG LABTECH continuously works with all major reagent companies to develop protocols and to optimize instrument settings for their existing assays and their newest kits.

Visit BMG LABTECH's Applications Center online to download all the leading applications, listed as:

- Application notes
- Scientific posters
- Peer-reviewed papers

BMG LABTECH's comprehensive searchable applications database reflects more than 20 years of expertise and innovations in microplate reading technology. Over 2.500 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 well trained distributors. Customers can rely on PhD level support and assistance with regard to software, assay development, or general enquiries related to the NEPHELOstar *Plus* and all other BMG LABTECH microplate reading solutions.

^{1,2,3} The graphs were taken from BMG LABTECH's Application Notes AN 117, AN 174 and we kindly thank Dr. Nelius Swart for the antigen-antibody binding figure.

NEPHELOstar^{Plus} - 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 Mode	Nephelometry, light-scattering
Measurement Modes	Endpoint and kinetic measurements
Microplate Formats	Up to 384-well plates
Light Source	Self-monitoring laser diode Wavelength 635 nm Selectable beam width: 1.5 to 3.5 mm Selectable intensity 0 to 100 % Scattering angle: Detects up to 80° full cone angle
Sensitivity	Depends on particle size and liquid properties Silica detection: 800 nM (particle size 0.5 to 10 µm) Dynamic range: 5 decades Maximum count rate (2,000,000 Relative) Nephelometric Units (RNUs) per second
Read Times	Depends on assay conditions and liquid surface stability 16 s (96-well), 47 s (384-well) (shortest possible times)
Reagent Injection	Up to 2 built-in reagent injectors Injection at measurement position Individual injection volumes for each well (3 to 500 µL) Variable injection speed up to 420 µL/s
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
Software	Multi-user software package including Reader Control and MARS Data Analysis Software, FDA 21 CFR part 11 compliant
Dimensions	Width: 44 cm, depth: 48 cm, height: 32 cm; weight: 25 kg
Accessories	
Atmospheric Control Unit (ACU)	Independently regulates both O ₂ (1-19 %) and CO ₂ (0-20 %) through a microprocessor control panel Dimensions (ACU only): Width: 44 cm, height: 17 cm and depth: 19 cm; Weight: 5 kg
Stacker	Mid-Throughput Microplate Handling System Magazines for up to 50 plates Continuous loading feature Barcode reader
THERMOstar	Microplate Incubator and Shaker Shakes and incubates four microplates simultaneously Incubation up to 56°C in 1°C steps Heating plates above and below the microplates
Upgrades	Upgrades to include options such as reagent injectors, gas vent, ACU 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: $3 \times (SD_{blank}) / \text{slope}$. Specifications are subject to change without notice.

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