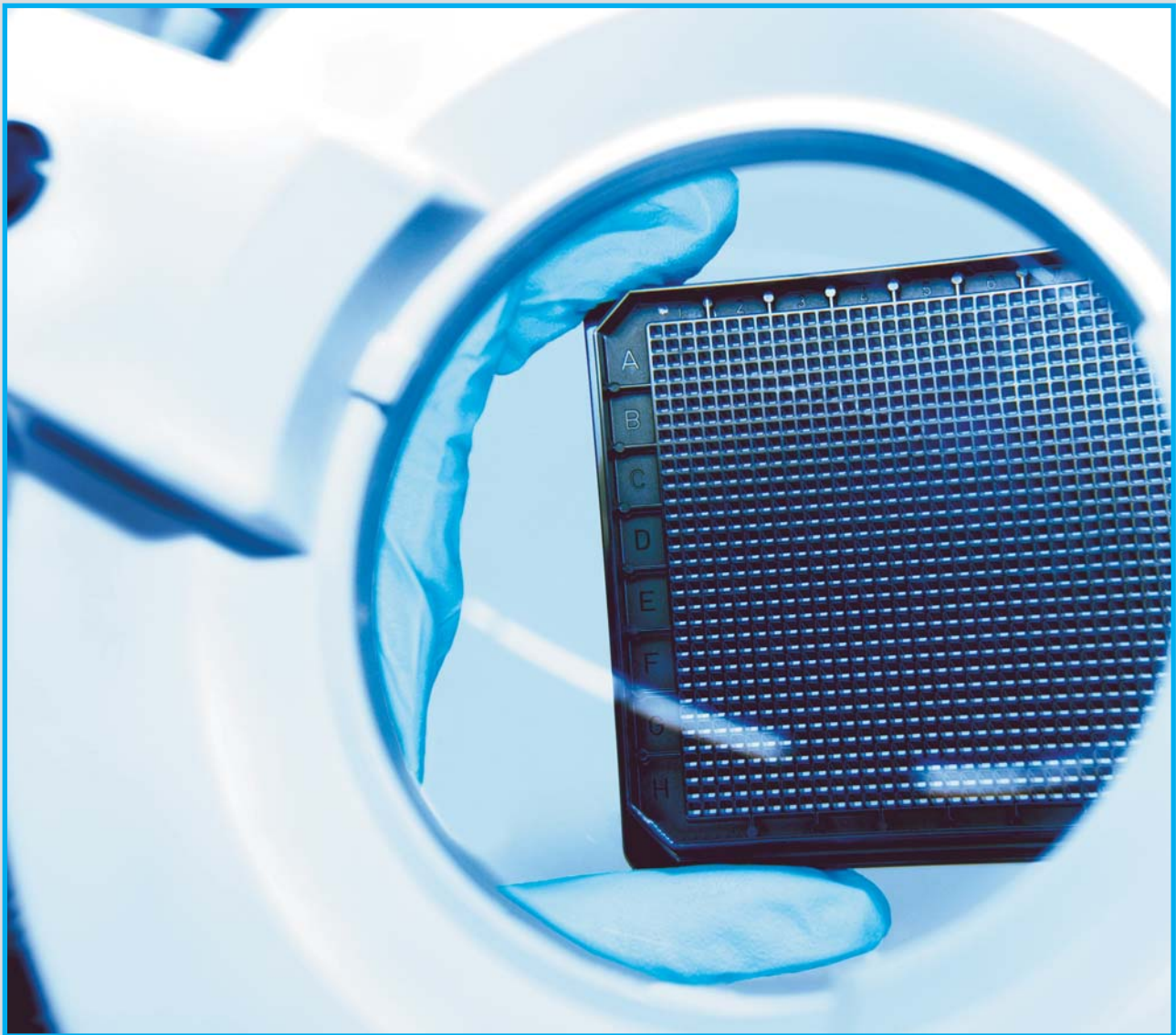


# High-Throughput Screening

Quality Control at Greiner Bio-One



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## Quality at Greiner Bio-One

Quality is the most important criteria in the manufacturing of our products. To ensure this is maintained at the highest level, we have a control system which monitors the physical, chemical and biological quality of our products. Quality checking starts with the incoming raw material, is continued in production processes and completed with the dispatch of the finished product. Strict controls, conducted according to the legal provisions and specific standards, accompany the product. Ongoing training in quality matters ensures that our employees are informed about the applications of our products and the quality requirements placed on them.

All products can be traced back to their production period, machine operators and tools, all the way back to the starting materials used.

With each delivery of goods, our customers receive a quality certificate and a guarantee of performance which commits us to maintaining our high quality standards.





# Certified Quality Standards

## Certification

Greiner Bio-One has been certified according to DIN EN ISO 9001 since 1994 and was recently re-certified in accordance with the new version of this standard DIN EN ISO 9001:2000 (Fig. 1). Since 2004 Greiner Bio-One has also been certified according to DIN EN ISO 13485:2003.

Several additional products are certified in accordance with EU-directives for medical products and In-Vitro Diagnostic devices.



Figure 1: DIN EN ISO 9001:2000 certification

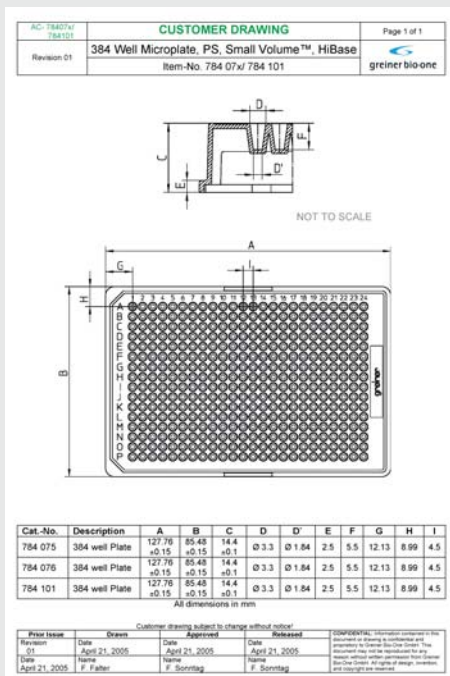


Figure 2: Customer drawing of a 384 well Small Volume™ HiBase microplate

## Documentation

Greiner Bio-One makes available additional product information to advise our customers on our quality standards.

- **Certificates**

Certificates describe the relevant quality criteria for each product and usually are included in the smallest packaging unit for most products.

- **Data sheets**

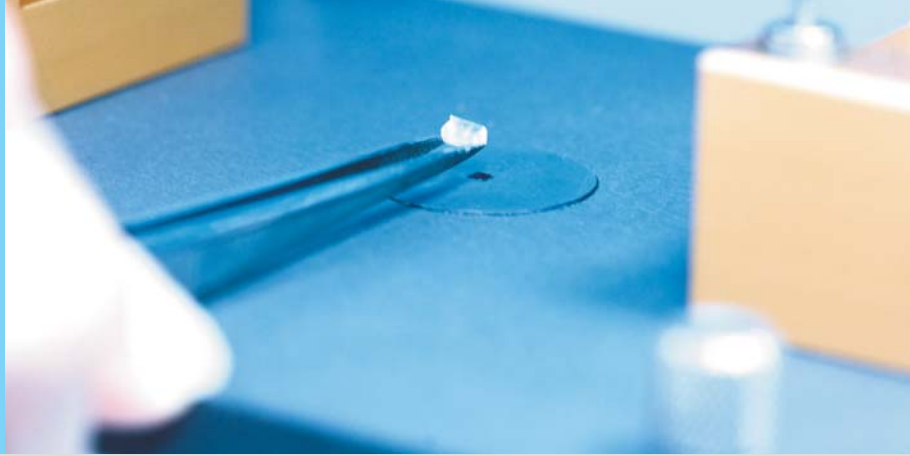
Data sheets contain a brief description of the product and its most important features. They serve for internal documentation in quality assurance, but may also be requested by customers as additional product information.

- **Customer drawings**

Customer drawings are available for all microplates. The drawings contain all important dimensions necessary for the calibration of liquid handling instrumentation or plate readers (Fig. 2).

The most important drawings and data sheets for microplates are available for download from our website.

# Raw Material Quality

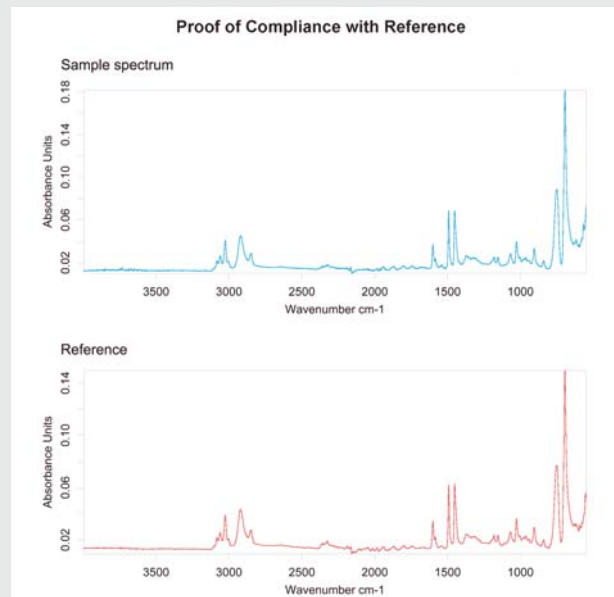


## Plastic Granulate

The plastic granulate of polystyrene and polypropylene used for microplates is characterised by high transparency and constant, reproducible quality.

All raw materials used in production are tested in our quality laboratory with regard to their suitability for the manufacture of microplates. A new type of raw material is only approved after undergoing extensive physical, chemical and biological testing. Every microplate produced will have specific, highly defined raw materials assigned to it, and only these can be used in its manufacture.

The plastic granulate as well as its composition is defined in these specifications for raw materials. Only those materials that conform to our specifications can be used to manufacture a product. Every batch of raw materials received is supplied with a manufacturer's guarantee that certifies the material complies with our specifications.



**Figure 3:** FT-IR spectrometry: Comparison of reference and sample spectra.

The quality of the raw materials is regularly monitored in a test specifically standardised for Greiner Bio-One. Moreover, the identity of the raw material is verified for each batch by analysis with an FT-IR spectrometer.

The calculated IR-spectrum is compared to an IR-spectrum of the original raw material type (Fig. 3) and has to meet the internal acceptance level.



Raw material analysis with an IR spectrometer

## Film Bottom

Film bottoms, which are used for  $\mu$ Clear<sup>®</sup> and UV-Star<sup>®</sup> products, must satisfy the same stringent quality guidelines as the plastic granulate. Specifications define our requirements and the quality of the film is controlled by regular testing.

Thus, for example, the signal-homogeneity, the background and the transparency of microplates with film bottoms are regularly monitored with optical measurements (Fig. 4).

Here the film bottoms must fulfil the following criteria:

- Autofluorescence of the films in the UV-range (< 400 nm) must remain constant from lot to lot
- The films must be optically transparent for microscopy

Additionally, the films must fulfil the following special requirements:

### $\mu$ Clear<sup>®</sup> microplates

The  $\mu$ Clear<sup>®</sup> film bottoms are made of polystyrene. They have, unless otherwise specified, a film thickness of 190  $\mu$ m +/- 15  $\mu$ m for 384 well and 96 well microplates, and 75  $\mu$ m +/- 15  $\mu$ m for 1536 well microplates.

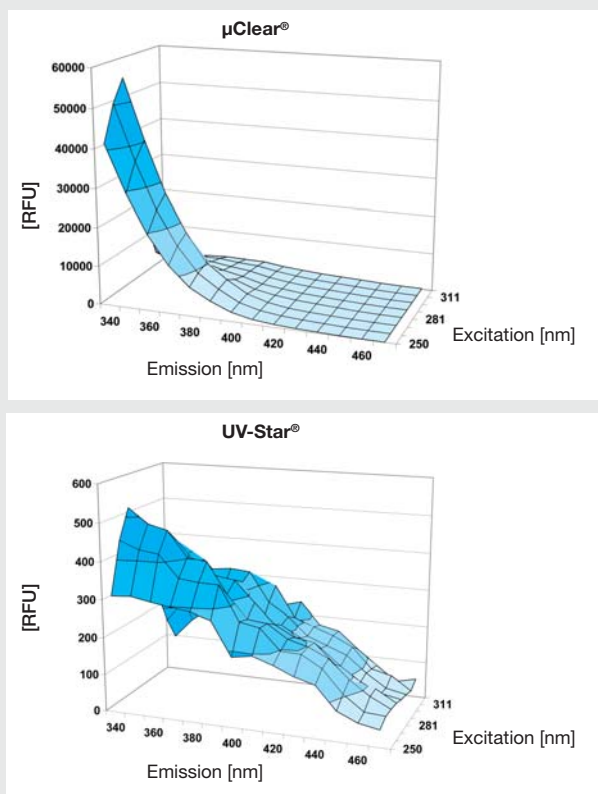


Figure 4: Optical measurement of background fluorescence of a  $\mu$ Clear<sup>®</sup> and a UV-Star<sup>®</sup> microplate

The optical density at 450 nm must lie, on average, under an extinction of 0.05. The coefficient of variation (CV) over a microplate at 450 nm must be less than 5%.

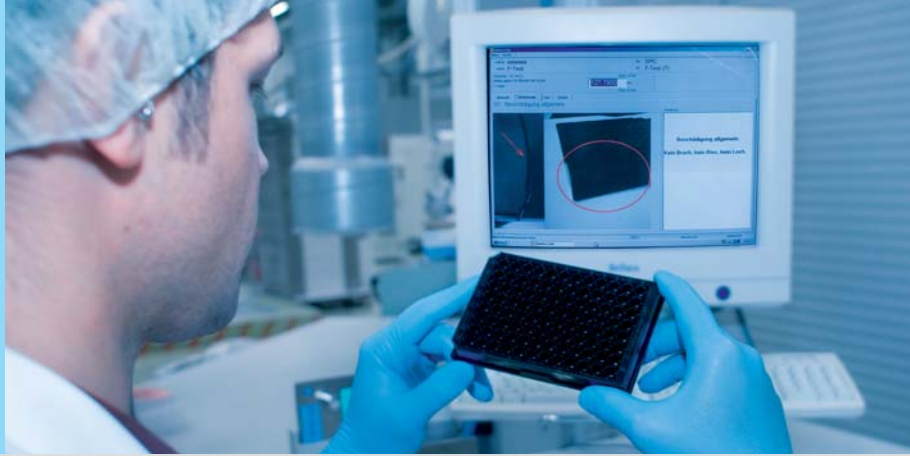
### UV-Star<sup>®</sup> microplates

The UV-Star<sup>®</sup> film bottoms are made of highly transparent polyolefin. They have a film thickness of 135  $\mu$ m +/- 10  $\mu$ m. On average, the optical density at 260 nm has to be less than 0.06. The CV over a plate at 260 nm must be less than 5%.



Determination of the optical quality of film bottom microplates in a microplate reader

# Microplate Dimensions



## Dimension Standards

Uniform and reproducible dimensions and tolerances are among the most important quality criteria for a microplate when used in a high-throughput screening regime.

All microplates manufactured by Greiner Bio-One have a uniform footprint (Fig. 5) which conforms to the recommendations of the American National Standards Institute (ANSI) (ANSI/SBS 1-2004). For further information about the ANSI/SBS Standards, please visit the Society's website: [www.sbsonline.org](http://www.sbsonline.org).

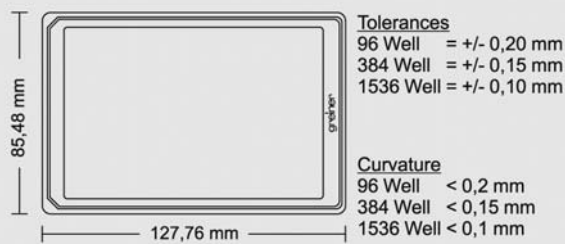


Figure 5: Footprint and tolerances of standard microplates

## Computer-aided Quality Assurance

All new products are carefully validated to fulfil our requirements. Additionally, the most critical dimensions and optical inspection criteria are routinely monitored during the manufacturing process for a defined number of samples and a defined inspection interval. This control is made throughout the running production process and supervised with a computer-aided quality assurance (CAQ) system (Fig. 6).

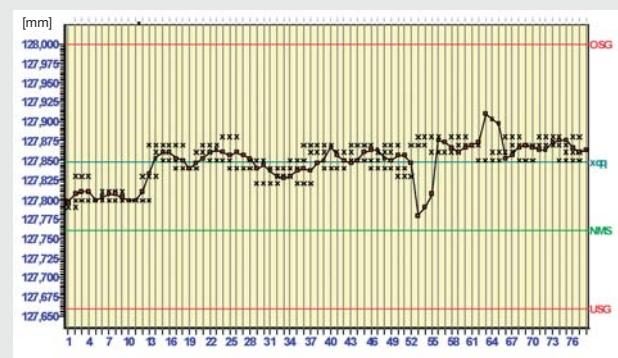


Figure 6: Monitoring of microplate length during production inspection.

The benefit of a CAQ system is the graphic display of all dimensional and optical test criteria. For every test it is clearly shown what is to be tested and how, and what the given tolerance limits are. If the tolerance limits are exceeded, the system automatically reports the event and initiates corrective measures.

With the data of the CAQ system, process capability can be monitored and evaluated. Several statistics can be used to measure the capability of a process:  $C_p$  and  $C_{pk}$  are process capability estimates. They show how capable a process is of meeting its specification limits, compared with continuous data.



Contact-free well geometry inspection of a microplate in a 3D coordinate measuring machine



# Immunological Quality

## Binding Properties

We set a high standard on the quality of our immunological products, especially on consistency and reproducibility of binding properties. The raw material used for immunological products is routinely tested for identity and immunological quality.

The immunological determination of biologically active substances is a well established part of modern analysis and laboratory medicine.

## Raw Material Quality

As the raw material used has a major influence on the binding properties of the final product, every incoming raw material batch is monitored as part of a sample production.

The immunological quality of the resulting sample plates is tested with an immunological assay (ELISA, LIA or FIA, depending on their applications) and must fulfil the following quality criteria:

- For intra-plate homogeneity the coefficient of variation (CV) must not exceed 5% for colorimetric or 10% for fluorescence and luminescence assays.
- For all immunological products, to guarantee constant binding properties, the CV for five tested plates must not exceed 10%. Additionally, the ratio of new sample plates to reference plates has to be in the range of 100 +/-10%.

The main criterion for our immunological products is a stable coefficient of variation (CV) from batch to batch which is monitored over a long period (Fig. 7).

If the criteria have been met, the raw material batch is approved and released for the production of immunological products.

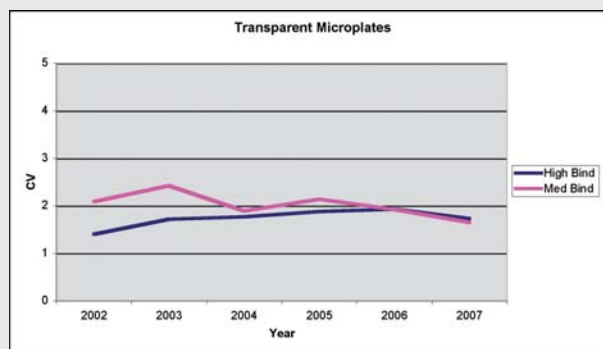


Figure 7: Coefficient of variation (CV) of all tested raw material batches from 2002 to 2007 for transparent microplates (med. and high binding)

This raw material batch is also documented on the package labelling of the end product. The package labelling of our immunological products is as follows:

The number of the raw material batch used can be found on the package box, alongside the weekly lot number, a consecutive box number and an in-process control number (Fig. 8).

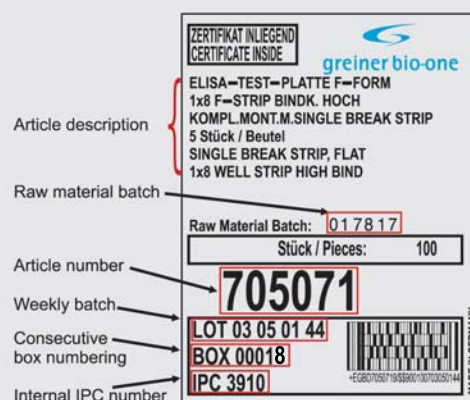


Figure 8: Package labelling of immunological products

# Biological Quality



## Human DNA, DNase, RNase

As microplates from Greiner Bio-One are used in various fields of biological applications, no endotoxin contamination or the presence of human DNA, DNases (deoxynucleases) or RNases (ribonucleases) is acceptable. These are found in all biological materials (hair, skin, nails, etc.), and both nucleases and DNA are extremely resistant to environmental influences.

Contamination with nucleases and DNA due to contact with human and animal material must therefore be prevented. Our production environment is designed in such a way as to minimise the risk of contamination.

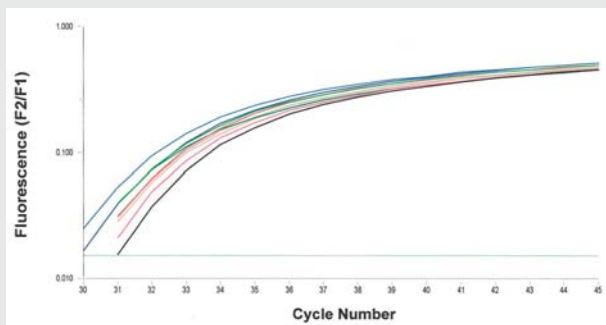
- All production staff wear overalls, gloves and hairnet, in order to prevent direct contact of the products with human material.
- The ambient air in the production area is strictly controlled by appropriate measures and is monitored for particles and microorganisms.

Spot-checks for contamination are performed throughout the year. Contamination with human DNA, DNase or RNase are tested as follows:

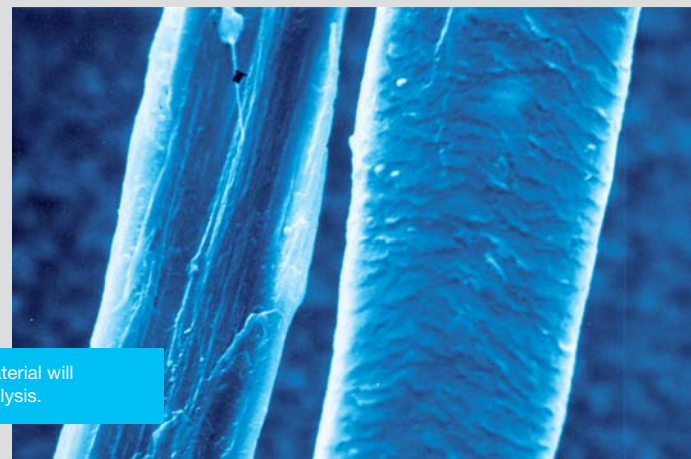
- The presence of DNase and RNase is tested with a test system based on the cleavage of a fluorescently labelled substrate (Fig. 9).
- The presence of genomic DNA (gDNA) is tested by quantifying the gDNA in a real time PCR, with a positive control (DNA standard) and a negative control (0.5% Tween20) included in each test.

! The detection limits for all assays are:

- RNase:  $5 \times 10^{-9} \text{ U}/\mu\text{l} = 5 \times 10^{-8} \text{ U/well} = 0.05 \text{ pg RNase}$
- DNase:  $1 \times 10^{-5} \text{ U}/\mu\text{l} = 1 \times 10^{-4} \text{ U/well} = 0.2 \text{ pg DNase}$
- gDNA:  $10 \text{ pg}/\mu\text{l} = 0.25 \text{ ng/well} = 15 \text{ copies}$



**Figure 9:** The presence of DNase is tested based on the cleavage of fluorescently labelled DNA. The chart shows a continuously present and stable fluorescence as proof of no contamination in the tested samples.



Contamination with biological material will generate false results in DNA analysis.

## Endotoxin

Contamination with endotoxins is tested with the Limulus amoebocyte lysate test (LAL). The test utilises the blood cells from the horseshoe crab (*Limulus polyphemus*) (Fig. 10), which react with endotoxins to produce a gelling of the lysate. This phenomenon confirms the presence of endotoxins in the sample and standard curves are used to determine the sensitivity of the test system.

The U.S. Pharmacopoeia stipulates that all medical products must not exceed the limit value of 0.5 EU/ml. Products that come into contact with cerebrospinal fluid must not exceed  $\leq 0.06$  EU/ml. Contamination with endotoxins due to contact with human and animal material must therefore be prevented.

- ! All Greiner Bio-One products tested are free from endotoxins up to a detection limit of  $\leq 0.06$  EU/ml (EU=endotoxin units). The test system meets the FDA guidelines for medical products (12/87).



**Figure 10:** Endotoxins are detected unequivocally by using the blue blood of the horseshoe crab (*Limulus polyphemus*).

## Sterility

In the sterilisation process, the process of microbial eradication is described by an exponential function. Therefore, the presence of microorganisms on any individual item can be put in terms of probability. The probability can be expressed as a sterility assurance level (SAL).

- ! The sterility of our products is guaranteed to SAL  $10^{-3}$  and represents the probability of there being a maximum of one non-sterile product unit present per 1000.

The validation of the necessary radiation dose is conducted in accordance with EN 552, ISO 11137 and ANSI/AAMI ST31/ST32.

On the basis of this standard, the so-called bioburden (germ level) is established for each product and the necessary radiation dose to attain the desired SAL can be determined. This determination is repeated at regular intervals.



Fully automated production site with strictly controlled manufacturing conditions

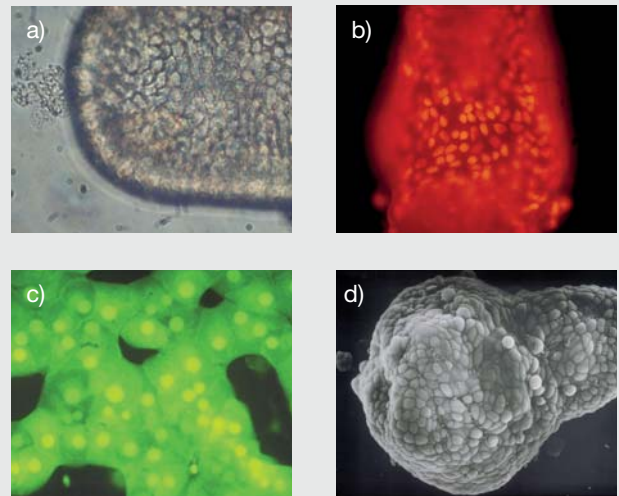
# Cell Culture Quality



## CELLSTAR® Quality

Greiner Bio-One offers a wide selection of products treated for cell culture (TC-treated microplates) to be found grouped together under the trademark CELLSTAR®.

Adherent cell lines require a polar surface with hydrophilic functional groups in order to adhere and grow. For polystyrene, this surface modification is achieved using a physical treatment process. Only a uniform surface treatment promotes stable and reproducible cell growth in the microplate, and this has to be constant from microplate to microplate and from lot to lot. Every modification, or the establishment of a new treatment process is evaluated through extensive physical and cell biological tests.



**Figure 11:** Microscopic images of cells

- a) Tissue section of liver
- b) Kidney cells stained with fura red for intracellular calcium monitoring
- c) Hepatocytes stained with BCECF for intracellular proton monitoring
- d) Spheroid cell culture of kidney cells

Once a treatment process has fulfilled all the requirements, it is approved for production. Thereafter the stability of the treatment is continually controlled by monitoring random samples.

! The surface quality is controlled by commonly used cell lines (e.g. CHO, chinese hamster ovary) and a highly sensitive cell line (SK-N-MC, human neuroblastoma).

We are proud of our high quality in cell culture treatment, which alongside the outstandingly uniform dimensions of our products, represents the second pillar for the success of our microplates.



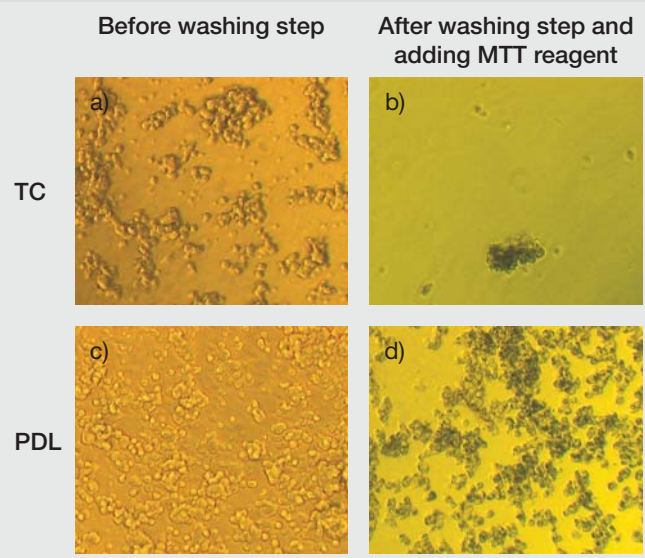
Microscopic inspection of cell growth in our cell culture treated microplates

## CELLCOAT® Quality

Greiner Bio-One's CELLCOAT® product range consists of biologically coated microplates and cell culture flasks. Through the biological coating of polystyrene surfaces with synthetic polypeptides and natural proteins, the adherence of sensitive cells can be significantly increased. Thereby cultivation of sensitive cell lines and the use of these cell lines in high-throughput screening can be achieved (Fig. 12).

Poly-D-Lysine (PDL), Poly-L-Lysine (PLL) and Collagen Type I are the most frequently used biopolymers for coating microplates. With PDL and PLL the cultivation efficiency of individual cell lines can be improved especially when serum-free or serum-reduced medium is used or when experiments such as transfections are performed. Collagen Type I is a protein of the extracellular matrix, an intercellular substance, which *in vivo* influences adhesion, migration and proliferation among other processes.

! Greiner Bio-One offers a diverse range of PDL, PLL and Collagen Type I coated microplates. Laminin and Fibronectin coated microplates are available upon request.



**Figure 12:** A neuroblastoma cell line cultivated on cell culture treated (TC) microplates resp. cultivated on PDL-coated microplates before (a, c) and after (b, d) a washing step.

The coating process takes place under the strictest quality standards in the clean room. Lotwise monitoring of the coated microplates for biological functionality and sterility as well as a standardised process guarantees the highest quality for our CELLCOAT® products.



For further information, please visit our website [www.gbo.com/bioscience](http://www.gbo.com/bioscience) or contact us:

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