forum

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Technical Notes and Applications for Laboratory Work

Contents

Enzyme linked immunosorbent assay microplates made of polylactate: properties, advantages, areas of application

Immunological test procedures, especially enzyme linked immunosorbent assays (ELISAs), are usually performed in microplates made of polystyrene in routine laboratory practice.

For many years, Greiner Bio-One has offered its tried and trusted MICROLON® product range for this purpose. The surface of a non-modified microplate made of polystyrene (MICROLON® 200 products) is hydrophobic and binds above all hydrophobic molecules. In order to bind hydrophilic molecules, the microplates have to be modified in a physical or chemical process (MICROLON® 600 products).

Despite comprehensive testing by a range of different microplate manufacturers or manufacturers of polystyrene batches, the ELISA results remain unsatisfactory for certain proteins. Alternatives to the classical polystyrene microplates were not available until recently.
ELISA 96 well microplates in 12 x 8-strip format from Greiner Bio-One made of the novel material polylactate should provide relief here and open up new test options in the immunological testing of peptides, proteins or conjugates. Polylactate (PLA) is the polymer of lactic acid.

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It is synthesised via the dimeric intermediate product lactide and can be technically processed like a plastic, similarly to polystyrene. In contrast to polystyrene, polylactate is completely biologically degradable and can be obtained from regenerative raw materials.
The stability of polylactate meets ELISA requirements. Under the influence of high temperatures (> 60 °C) or high humidity (> 60 %), polylactate is slowly broken down autocatalytically.

Properties, advantages, areas of application

1. Background and coefficient of variation in polylactate microplates

The binding capacity of a polymer surface is determined firstly by the chemical structure, and secondly by the surface properties of the matrix. These are in turn dependent on the manufacturing process.
Polylactate can be processed at low temperatures. This minimises the danger of temperature fluctuations during production. This and the polyester chemistry of the material produces a very low background and coefficient of variation (CV) during tests (Fig. 1, CV< 2 %).

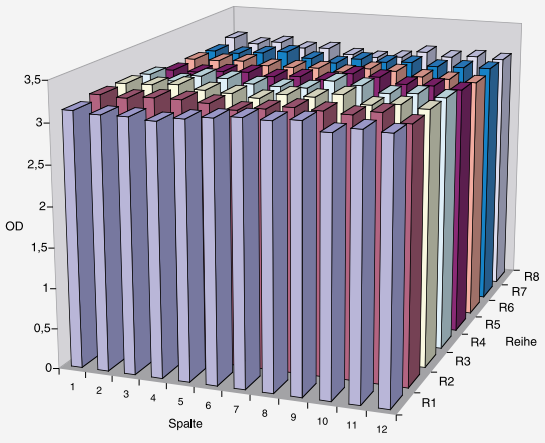


Figure 1:
Coefficient of variation in a 96 well polylactate microplate CV = 1.6)

2. Extended standard curve range

Many ELISA systems are based on microplates coated with antibodies. The quantification of the protein or hapten to be detected is done on the basis of a comparison with a standard curve, which should as far as possible encompass a broad measuring range.

In order to determine the standard curve range of polylactate microplates on the basis of immunoglobulin G, purified immunoglobulin fractions dissolved in PBS were adhesively bound to polystyrene and polylactate surfaces in comparative test series. The results (Fig. 2) show that proteins can be detected with polylactate microplates in the upper standard curve range and definitely up to 5 - 10 µg. In the lower range, depending on the quality of the specific immunoglobulin, detection limits of up to 5 ng protein per well are achieved.

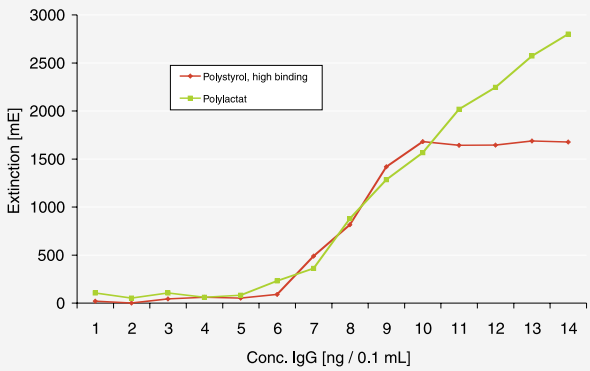


Figure 2:
Standard curve (IgG) in a 96 well polystyrene and 96 well polylactate microplate

3. Influence of the pH value on protein binding

In the coating of polylactate microplates with different proteins, here using the example of immunoglobulin G, it is found that comparable amounts of protein are bound in the range between pH 4.5 and pH 9.5 (Fig. 3). Polylactate microplates do not show any limitations with regard to pH value. This is advantageous especially for proteins or conjugates that can only be dissolved in the weakly acidic or

basic range.

In order to guarantee homogeneous binding in the wells of the microplates, they have to be coated at low pH values.

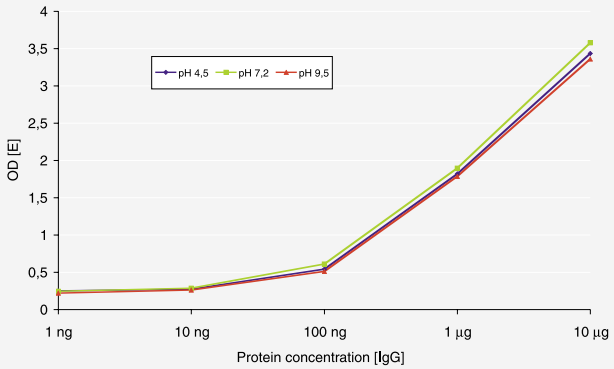


Figure 3:
Influence of the pH value on protein binding (IgG) in 96 well polylactate microplates

4. Detection of low molecular haptens

Low molecular haptens are difficult to detect in enzyme immunological test procedures. The low molecular weight often prevents the detection limits from being reached. The performance capacity of a test system is therefore especially easy to show using low molecular haptens.
In a comparative test on polystyrene and polylactate microplates, the commonly available pesticide MCPA (molecular weight 200.62 g/Mol) was investigated as a hapten.
Comparison of the standard curves (Fig. 4) shows that smaller amounts of hapten can be detected on polylactate plates with lower background.

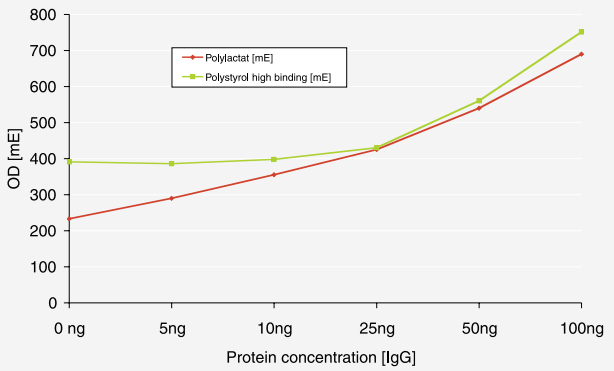


Figure 4:
MCPA detection in 96 well polylactate and 96 well polystyrene microplates

5. Technical data

In order to successfully work with polylactate microplates, it is usually not necessary to change standard ELISA protocols.
To achieve reproducible results, coating overnight and the use of PBS buffer instead of the usual PBA buffer is recommended. In the case of colorimetric tests, it should be taken into account that the changing colour takes place more slowly in polylactate microplates than in polystyrene microplates.

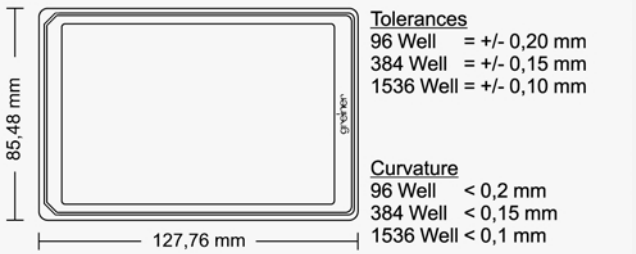
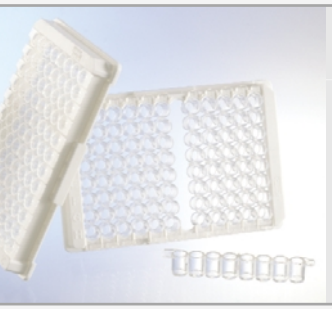


Figure 5:
External dimensions and tolerances of a standard microplate

Ordering Information

	Cat.-No.	Format	Description	Qty. per bag/case
	762 870	**96 well**	Strip-Plate, 12 x F8 Strips, F-bottom, Polylactate, transparent	5/100

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Netherlands: (+31) 172-42 09 00 · info@nl.gbo.com, **UK:** (+44) 14 53-82 52 55 · info@uk.gbo.com, **USA:** (+1) 8 00-8 84-47 03 · info@us.gbo.com