Immunology

ELISA (Enzyme-Linked Immunosorbent Assay) is probably the most widely used biochemical method in laboratory analysis and diagnostics. Analytes such as peptides, proteins, antibodies and hormones can be detected selectively in low concentrations among a multitude of other substances and be quantified. Additionally, ELISAs are rapid, sensitive, cost-effective and can be performed in a high-throughput manner.

ELISA is used in a variety of different assay types (e.g. direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA). Nevertheless, all ELISA variants are based on the same principle, the binding of one assay component – antigen or specific antibody – to a solid surface and the selective interaction between both assay components. Molecules not specifically interacting with the assay component bound to the solid surface are washed away during the assay.

For detection of the interaction the antibody or antigen is labelled or linked to an enzyme (direct ELISA; Fig. 1). Alternatively, a secondary antibody conjugate can be used (indirect ELISA; Fig. 2). The assay is processed by adding an enzymatic substrate to produce a measurable signal (colorimetric, fluorescent or luminescent). The strength of the signal indicates the quantity of analytes in the sample.

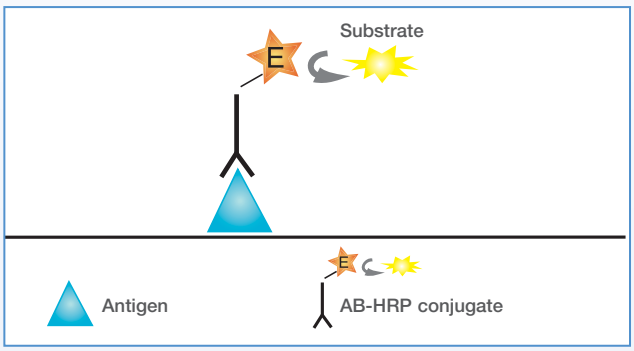

Figure 1: Direct ELISA

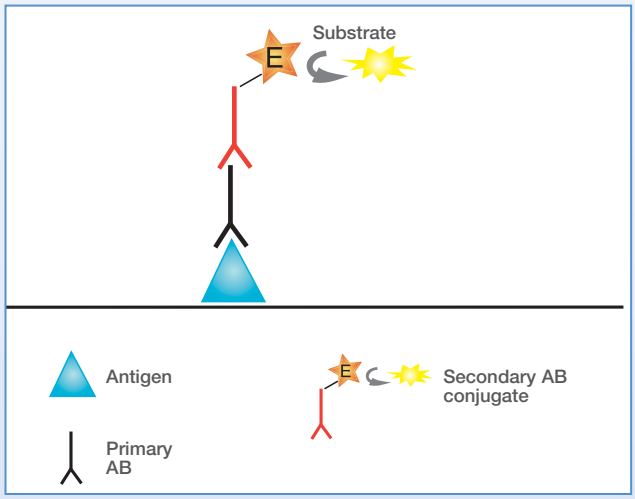

Figure 2: Indirect ELISA

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Further information on ELISA

→ **Forum No. 9: Microplates for enzyme-linked immunosorbent assays (ELISA)** (F073 004)
→ **Application Note "Insulin ELISA on high binding MICROLON® 600 and CELLSTAR® microplates"** (F073 106)
→ **Application Note "Influence of coating buffer and incubation conditions on ELISA performance"** (F073 118)

Surface Properties and Microplate Colour

A key step in ELISA is the binding of one assay component – antigen or antibody – to the solid surface by passive adsorption. Therefore, the features of this surface are crucial for the performance of the assay.
All ELISA microplates from Greiner Bio-One are made out of high-quality virgin polystyrene. The resin is highly transparent and therefore ideally suited for optical measurements. Due to its chemical nature polystyrene is a hydrophobic compound. Hydrophilic groups can be introduced to polystyrene surfaces by physical treatment. Greiner Bio-One offers two surface qualities for ELISA microplates: the hydrophilic **high binding** products and the less hydrophilic **medium binding** products.

Since attachment to a solid surface based upon passive adsorption depends as well on the properties of the molecule to be bound, it is therefore advisable to compare the performance of high binding and medium binding products when developing a new assay.

Beside products made of clear polystyrene for colorimetric measurements, Greiner Bio-One offers a wide variety of black and white ELISA microplates for luminescence and fluorescence measurements. Colour and surface properties can be deduced from the respective brand name of our products (Table 1).

Brand name	Surface property	Colour
MICROLON® 200	Medium binding	clear
MICROLON® 600	High binding	clear
FLUOTRAC™ 200	Medium binding	black
FLUOTRAC™ 600	High binding	black
LUMITRAC™ 200	Medium binding	white
LUMITRAC™ 600	High binding	white

Table 1: Assignment of brand names and properties of ELISA microplates

Quality aspects

We set high standards on the quality of our immunological products, especially on consistency and reproducibility of binding properties. As the raw material has a major influence on the binding properties of the final product, the incoming raw material used for ELISA microplates is routinely monitored for identity and immunological quality. Sample plates are tested with an immunoassay (ELISA, LIA or FIA, depending on their applications) and must fulfil the following 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 provide 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 ELISA microplates is a stable coefficient of variation (CV) from batch to batch which is monitored over a long period (Fig. 3).

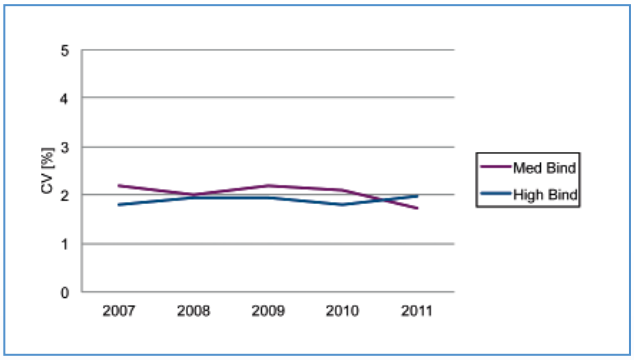

Figure 3: Coefficient of variation (CV) of tested raw material batches from 2007 to 2011 for transparent microplates (med. and high binding)

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

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

The number of the raw material batch used can be found on the package box, alongside the shelf life, the lot number, a consecutive box number and an in-process control number (Fig. 4).

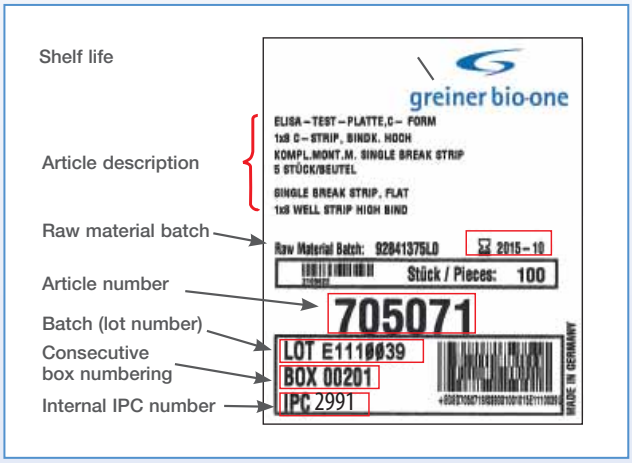

Figure 4: Package labelling of immunological products