



The *recoveryELISA* is a patented development of BioTeZ.

**Classic antibody-based measurement methods in the presence of therapeutic antibodies are significantly disturbed when the measurement of the target molecule (antigen) of therapeutic antibody is to be determined. *recoveryELISA* is a diagnostic solution and provides a sample measurement of three results:**

- The concentration of free TNF- $\alpha$  antigen
- The concentration of available therapeutic antibody as well as
- A measurement reading reveals the interaction between antigen and therapeutic antibody levels in terms of a dose-response relationship

BioTeZ tested the *recoveryELISA* as diagnostic method for many Biologics successfully. In the field of Anti-rheumatism or for treatment of Sarcoidosis with anti-TNF- $\alpha$  therapeutic antibodies are available Kits only for research, Test kits for further Biologics and their Antigens are under development:

- ***recoveryELISA* Kit free TNF- $\alpha$ / available Adalimumab**
- ***recoveryELISA* Kit free TNF- $\alpha$ / available Infliximab**

It is expected that these test kits will be available as In-vitro-Diagnostic with CE mark in April 2012.

BioTeZ takes on the measurement of serum samples for research. Test kits for determination of 7 samples:

- For the analysis of serum samples, 0.5 ml and 0.15 ml would be perfectly adequate.
- Duplicate determination of each sample
- Minimum quantity: 3 samples
- Price: 85,00 Euro plus VAT per sample

#### Methodology

RecoveryELISA (Enzyme-Linked Immunosorbent Assay) is an immunological quantitative detection method based on a sandwich ELISA. In comparison to a classic sandwich ELISA, a two-dimensional calibration is carried out for recoveryELISA obtaining two analysis results within the same assay. The following calibrations are performed:

1. Antigen calibration without and with additional therapeutic antibody (TAB) against extinction (optical density)
2. TAB calibration against antigen recovery

RecoveryELISA is performed in a 96-well microplate. The wells of the microplate are pre-coated with a specific capture antibody. There occurs a simultaneous incubation of the antigen levels (with and without the addition of TAB) of the samples and of the detection conjugate (peroxidase conjugate of an anti-antigen antibody). Incubation takes place over 16 to 22 h at 2 to 8°C. After washing, the colour substrate TMB (tetramethylbenzidine) is added to the wells. After incubation the enzymatic colour reaction is stopped using a sulphuric acid solution. A colour change from blue to yellow occurs that is optically detected. The optical density (OD) of the reaction product is measured with a suitable microplate reader.

With the aid of the two calibrations to be performed, an evaluation procedure can be carried out to determine the concentrations of free antigen and available TAB in the patient samples using the measured OD values. Determination of the therapeutic antibody is based on the principle that the presence of TAB in patient samples leads to a systematic reduction in the recovery of antigen. The evaluation is performed using non-linear regression. Specific software is available.

**For further information please have a look on our homepage.**