

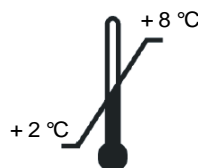
# TNF $\alpha$ -Blocker-ADA (anti-drug-antib. e.g. Enbrel) ELISA Kit

*For the in vitro determination of human antibodies against  
drugs in the rheumatism therapy (e.g. Enbrel®) in serum*

Valid from 14.01.2010



IMM-K 9653



## 1. INTENDED USE

The here described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for the qualitative determination of human antibodies against drugs in the rheumatism therapy (e.g. Enbrel®) in serum. It is for *in vitro* diagnostic use only.

## 2. CLINICAL RELEVANCE

With our ELISA test, antibodies against drugs in the rheumatism therapy (e.g. Enbrel®) can be detected.

Drugs in the rheumatism therapy (e.g. Enbrel®) are used to suppress inflammation in rheumatic patients. During the therapy with drugs (e.g. Enbrel®), some patients can develop antibodies against the drug itself. This might lead to severe complications, even a systemic anaphylaxis with possibly lethal outcome.

Our ELISA kit can be used for monitoring anti-drug (e.g. Enbrel®) antibodies during therapy and offers the doctor a tool for decision on possible preventive measures.

## 3. PRINCIPLE OF THE TEST

This Enzyme-Immuno-test is a sandwich assay for the determination of anti-drug (e.g. Enbrel®) antibodies in serum samples. During the first incubation step, the drug immobilized on the wall of the microtiter wells captures the anti-drug antibodies in the patient samples. After washing away the unbound components from samples, a Peroxidase-labelled conjugate is added to each well. After a second washing step, tetramethylbenzidine (TMB), a peroxidase substrate, is added. Finally, the reaction is terminated with an acidic stop solution. The intensity of the yellow color is directly proportional to the anti-drug antibody concentration of sample.

**4. MATERIAL SUPPLIED**

<b>Cat. No.</b>	<b>Label</b>	<b>Kit Components</b>	<b>Quantity</b>
K 9653MTP	PLATE	One holder with strips, precoated	12 x 8 wells
K 9653WP	WASHBUF	ELISA wash buffer concentrate 10x	1 x 100 ml
K 9653K	CONJ	Conjugate, (peroxidase labelled), concentrate	1 x 200 $\mu$ l
K 9653KO1	CTRL POS	Control, positive	4 x 1 vial
K 9653KO2	CTRL NEG	Control, negative	4 x 1 vial
K 9653PV	SAMPLEBUF	Dilution buffer, ready-to-use	2 x 100 ml
K 9653TMB	SUB	TMB substrate (Tetramethylbenzidine), ready-to-use	1 x 15 ml
K 9653AC	STOP	ELISA stop solution, ready-to-use	1 x 15 ml

**5. MATERIAL REQUIRED BUT NOT SUPPLIED**

- Bidistilled water (aqua bidest.)
- Precision pipettors calibrated and tips to deliver 5-1000  $\mu$ l
- Absorbent paper
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 or 405 nm  
(reference wave length 620 or 690 nm)

## 6. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100  $\mu$ l** should be centrifuged before use to avoid loss of volume.
- The **ELISA wash buffer concentrate** (WASHBUF) should be diluted with aqua bidest. **1:10** before use (100 ml concentrate + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C before dilution of the buffer solutions. The **buffer concentrate** is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month**.
- The lyophilized **CTRLNEG** and **CTRLPOS** (controls, negative and positive) must be reconstituted with **500  $\mu$ l** of **aqua bidest**. Allow the vial content to dissolve for 10 minutes at room temperature, and mix thoroughly by gentle inversion to insure complete reconstitution. The undiluted controls are stable at **2-8 °C** until expiry date stated on the label. **Reconstituted controls are not stable and can not be stored.**
- The **conjugate** (CONJ) must be diluted **1:100** in wash buffer (100  $\mu$ l CONJ + 10 ml wash buffer). The undiluted conjugate is stable at **2-8 °C** until expiry date stated on the label. **Diluted conjugate is not stable and can not be stored.**
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at **2-8°C**.

## 7. PRECAUTIONS

- For *in vitro* diagnostic use only.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water. Do not breath vapour and avoid inhalation.
- Reagents should not be used beyond the expiration date stated on kit label.

## 8. SPECIMEN COLLECTION AND PREPARATION

### Serum

Serum samples must be diluted **1:200** before performing the assay, e.g.

**5  $\mu$ l** serum + **995  $\mu$ l** SAMPLEBUF (dilution buffer), mix well.

**100  $\mu$ l** of the diluted sample per well are used in the test.

## 9. ASSAY PROCEDURE

### *Procedural notes*

- Do not mix different lot numbers of any kit component within the same assay.
- The quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the test results. Immundiagnostik can therefore not be held responsible for any damage.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Carry out the assay with the actual manual delivered with the kit.

## Test procedure

Wash the precoated microtiter plate **5 x with 250  $\mu$ l ELISA wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper. Carry out the tests in duplicate.

1. Add <b>100 <math>\mu</math>l</b> of CTRLNEG, CTRLPOS (controls) and diluted samples in the wells of the microtiter plate.
2. Incubate <b>for 2 hours</b> shaking on a horizontal mixer at room temperature (18–26°C).*
3. Aspirate the content of the plate and wash each well <b>5 x with 250 <math>\mu</math>l ELISA wash buffer</b> . After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
4. Add <b>100 <math>\mu</math>l</b> of diluted CONJ (conjugate) into each well.
5. Incubate for <b>1 hour</b> shaking on a horizontal mixer at room temperature (18–26°C).
6. Aspirate the content of the plate and wash each well <b>5 x with 250 <math>\mu</math>l ELISA wash buffer</b> . After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
7. Add <b>100 <math>\mu</math>l</b> of TMB substrate solution into each well.
8. Incubate for <b>5-15 minutes</b> at room temperature in the dark. **
9. Add <b>50 <math>\mu</math>l</b> stop solution into each well and mix shortly.
10. Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference.

\*The above incubation step at 18–26 °C on a horizontal mixer is recommended by the producer. If there is no possibility to incubate at 18–26 °C, while shaking, we recommend to incubate at 18–26 °C without any shaking.

\*\*The intensity of the color change is temperature sensitive. We recommend to observe the procedure of the color change and to stop the reaction upon good differentiation.

## 10. RESULTS

The results are evaluated by a cut-off value which is estimated by multiplying the OD of the negative control by 3.5.

## 11. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- The test components which are made of human serum are tested for HVB and HIV and found to be negative. However, since no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as recommended for any potentially infectious human serum or blood specimen. The normal precautions for laboratory working should be observed.
- Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- All reagents in the test package are to be used for *in-vitro* diagnostics only.
- The reagents should not be used after the date of expiry (see label on the test package). Single components with different lot numbers should not be mixed or exchanged.
- The guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components have been defined by the producer. Any alterations of the test procedure, that are not coordinated with the producer, may influence the results of the test. Immundiagnostik can therefore not be held responsible for any damage.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product shall be send to Immundiagnostik AG along with a written complaint.

**Used symbols:**



Temperature limitation



Catalogue Number



In Vitro Diagnostic Medical Device



Contains sufficient for <n> tests



Manufacturer



Use by



Lot number