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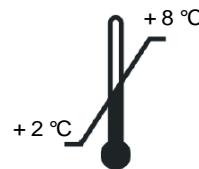
TNF α -Blocker-Monitoring (drug-level e.g. Humira) ELISA Kit

*For the in vitro determination of TNF α antibody concentration
(e.g. Humira) in serum*

For research use only

Gültig ab / valid from 03.11.2009

IMM-K 9657



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1. INTENDED USE

The *Immundiagnostik* Assay is a Enzyme Immuno Assay intended for the qualitative determination of anti-TNF α antibodies (e.g. Adalimumab/Humira) in serum. For research use only.

2. CLINICAL RELEVANCE

Tumour Necrosis Factor alpha (TNF α) belongs to the pro-inflammatory cytokines that establish and sustain inflammation reactions. Cytokines, produced by macrophages and T-cells, play a central role in both acute and chronic inflammations.

The TNF α concentration is elevated in the affected joints in many rheumatic diseases (rheumatoid arthritis, chronic poly-arthritis, ankylosing spondylitis i.e. M. Bechterew disease, psoriasis) and plays a significant role in joint destruction as well as in other manifestations of the diseases. Even in Crohn's disease, an overproduction of TNF α has been observed, obviously affecting the activity of the disease.

In the 1990's, pharmaceutical companies developed biotechnologically produced drugs that are aimed to neutralize TNF α ("TNF α blockers") and expected to have a positive effect on the various symptoms of the diseases.

In 1998, the first TNF α blockers were approved for use in the therapy of rheumatoid arthritis. Since then other TNF α blockers have been marketed:

- Infliximab (Remicade®),
- Adalimumab (Humira®).

The two TNF α blockers are approved for treatment of rheumatoid arthritis as well as ankylosing spondylitis and psoriasis.

Although the TNF α blockers differ in respect to their chemical structures and mechanisms of action, the pharmacological effects are the same for both substances. They are comparable in their effectiveness in facilitating improvement in clinical symptoms of rheumatoid arthritis, but in Crohn's disease, Infliximab has proved to be the most effective one.

The therapeutic effect of the anti TNF α antibodies on chronic inflammations, i.e. Crohn's disease or rheumatoid arthritis, depends on the serum concentration of the corresponding pharmaceutical. In many patients, the treatment is of limited value, because of a fast degradation of the pharmaceutical or generation of antibodies against it. For a better control of the therapy, drug level monitoring is necessary.

With our ELISA test, anti-TNF α -therapeutic antibodies can be detected. Anti-TNF α -antibodies are used for suppressing therapy in rheumatic patients.

Beside TNF α blocker degradation during therapy, patients under anti-TNF α -antibodies therapy could develop antibodies against the therapeutic antibodies. This might lead to severe complications, even systemic anaphylaxis with possibly lethal outcome.

Our ELISA test can be used for monitoring anti-TNF α -antibodies and provides a basis for possible preventive strategies.

3. PRINCIPLE OF THE TEST

This Enzyme Immuno Assay is a assay for the quantitative determination of anti-TNF α antibodies in serum samples. In a first incubation step, the antibodies from the sample are bound to the on the plate coated TNF α . To remove all unbound substances, a washing step is carried out.

In a further incubation step, Peroxidase-labeled antibody is added. After another washing step, to remove all unbound substances, the solid phase is incubated with the substrate, Tetramethylbenzidine (TMB). An acidic stop solution is then added. The color converts to yellow. The results are determined by a cut off.

4. MATERIAL SUPPLIED

Cat. Nr.	Label	Kit Components	Quantity
K9657MTP	PLATE	One holder with strips, precoated	12 x 8 wells
K9657WP	WASHBUF	ELISA wash buffer concentrate 10x	1 x 100 ml
K9657K	CONJ	Conjugate, Peroxidase-labeled, ready-to-use	1 x 12 ml
K9657ST	STD	Calibrators, lyophilized (0;0,625;1,25;2,5;5;10;20 μ g/ml)	7 x 1 vial
K9657KO	CTRL	Control, lyophilized	1 x 1 vial
K9657PV	SAMPLEBUF	Dilution buffer, ready-to-use	2 x 100 ml
K9657TMB	SUB	TMB Substrate (Tetramethylbenzidin), ready-to-use	1 x 15 ml
K9657AC	STOP	ELISA stop solution, ready-to-use	1 x 15 ml

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled (aqua bidest.) and sterile water
- Laboratory balance
- Precision pipettors calibrated and tips to deliver 5-1000 μ l
- 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

- Reagents with a volume less than **100 μ l** should be centrifuged before use to avoid loss of volume.
- The **WASHBUF** (wash buffer concentrate) must be diluted with aqua bidest. **1:10** before use (100 ml WASHBUF + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37°C using a water bath **before dilution of the buffer solutions**. The **WASHBUF** (wash 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 **STD** (standards) and **CTRL** (control) are stable up to the expiry date when stored at 2-8°C. The lyophilized **STD** (standards) and **CTRL** (control) must be reconstituted with **250 μ l** aqua bidest. Allow the vial content to dissolve for **10 minutes** and mix thoroughly by gentle inversion to insure complete reconstitution. **Reconstituted Standards and Control are not stable and can not be stored**.
- All other test reagents are ready for use. The test reagents are stable up to the date of expiry (see label of test package) when stored at 2-8°C.

7. PRECAUTIONS

- For research use only.
- The calibrators and controls contain human source material which was tested and found to be non-reactive to HBsAg, anti-HIV-1/2. Since no method can offer complete assurance that hepatitis B virus, HIV-1/2, HCV or other infectious agents are absent, these reagents should be handled as if potentially infectious.
- 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 **1:250** before performing the assay, e.g.

4 μ l serum + **996 μ l** SAMPLEBUF (sample dilution buffer), mix well.

100 μ 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.
- Carry out the assay with the actual manual delivered with the kit.

Test procedure

1.	Prior to use in the assay allow all reagents and samples to come to room temperature (18-26 °C) and mix well
2.	Mark the positions of STD (standards), CTRL (control) and SAMPLE (sample) on a protocol sheet
3.	Take as many microtiter strips (PLATE) as needed from kit. Wash the precoated microtiter plate 5 times by dispensing 250 μl of diluted wash buffer into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
4.	For the analysis in duplicate, pipette 100 μl of STD (standards), CTRL (control) and SAMPLE (samples) into the respective well of the microtiter plate
5.	Cover plate tightly and incubate for 4 hours at room temperature (18-26 °C)
6.	Aspirate the contents of each well. Wash 5 times by dispensing 250 μl of diluted wash buffer into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
7.	Add 100 μl of CONJ (conjugate) into each well
8.	Cover the plate tightly and incubate for 1 hour at room temperature (18-26°C) in the dark while shaking
9.	Aspirate the contents of each well. Wash 5 times by dispensing 250 μl of diluted wash buffer into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
10.	Add 100 μl of SUB (TMB substrate) into each well
11.	Incubate for 5-10 min at room temperature in the dark*
12.	Add 50 μl of STOP (stop solution) into each well, mix thoroughly
13.	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 intensity of the color change is temperature sensitive. We recommend to observe the color change and to stop the reaction upon good differentiation.

10. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the "4-Parameter-algorithm".

1. 4-Parameter-algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).

2. Point-to-point-calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline-algorithm

We recommend a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

11. LIMITATIONS

Samples with analyte levels greater than the highest calibrator should be further diluted and re-assayed.

12. QUALITY CONTROL

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

13. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay

The precision (intra-assay variation) was calculated from 20 replicate determinations on one sample.

Intra-Assay CV n=20

Sample	Anti TNF α -antibodies [μ g/ml]	Intra-Assay CV [%]
1	4,8	3,6

Sensitivity

The detection limit was set as $B_0 + 2 \text{ SD}$ and estimated to be 0,04 μ g/l. The Zero-standard was measured 20 times.

Cross reactivity

No cross reactivity to other plasma proteins was observed.

14. REFERENCES

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

15. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- 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.

- Reagents of the test package contain sodium azide as a bactericide. Contact with skin or mucous membranes has to be avoided.
- All reagents in the test package are for research use 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.

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Used symbols:

	Temperature limitation		Catalogue Number
	In Vitro Diagnostic Medical Device		Contains sufficient for <n> tests
	Manufacturer		Use by
	Lot number		For research use only

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