



Li StarFish S.r.l.
Via Cavour, 35 - 20063 Cernusco S/N (MI), Italy
Tel. +39-02-92150794 - Fax. +39-02-92157285
info@listarfish.it - www.listarfish.it

Anti-TNF α Blocker ELISA Kit

Zur in vitro Bestimmung von humanen Antikörpern gegen voll humane anti-TNF α Antikörpern (wie z. B. HUMIRA) in Serum

Anti-TNF α blocker ELISA Kit

For the in vitro determination of human antibody against completely human anti-TNF α antibody (e.g. HUMIRA) in serum

Nur zu wissenschaftlichen Zwecken / For research use only

Gültig ab/valid from 07.04.2005

Artikelnummer/Catalogue No.: IMM-K 9652

Packungsgröße/Package size: 96 Tests/96 determinations

Lagerung/Storage: 2 - 8 °C

Table of Content	Page
	2
1. INTENDED USE	12
2. CLINICAL RELEVANCE	12
3. PRINCIPLE OF THE TEST	12
4. MATERIAL SUPPLIED	13
5. MATERIAL REQUIRED BUT NOT SUPPLIED	14
6. PREPARATION AND STORAGE OF REAGENTS	14
7. PRECAUTIONS	15
8. SPECIMEN COLLECTION AND PREPARATION	15
9. ASSAY PROCEDURE	16
PROCEDURAL NOTES	16
TEST PROCEDURE	16
10. RESULTS	18
11. PERFORMANCE CHARACTERISTICS	18
PRECISION AND REPRODUCIBILITY	18
12. GENERAL NOTES ON THE TEST AND TEST PROCEDURE	19

1. INTENDED USE

The *Immundiagnostik* Assay is a sandwich Enzyme Immuno Assay intended for the qualitative determination of human antibody against completely human anti-TNF α antibody (e.g. HUMIRA) in serum.

2. CLINICAL RELEVANCE

With our ELISA test, antibodies against anti-TNF α -therapeutic antibodies can be detected.

Anti-TNF α -antibodies are for example used for suppressing therapy in rheumatic patients. There is a possibility that patients under anti-TNF α -antibodies therapy 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 therapy and offers the doctor an instrument for deciding on possible preventive measures.

3. PRINCIPLE OF THE TEST

This Enzyme Immuno Assay is a sandwich assay for the 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 therapy antibody (F(ab)₂). To remove all unbound substances, a washing step is carried out.

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

4. MATERIAL SUPPLIED

Catalogue No.	Kit Components	Quantity
K 9652MTP	one holder with strips, precoated (F(ab) ₂)	96
K 9652WP	ELISA wash buffer concentrate 10x	1 x 100 ml
K 9652K	POD antibody, (therapy antibody, peroxidase labelled), concentrate	1 vial
K 9652KO1	Control, positive	4 x 1 vial
K 9652KO2	Control, negative	4 x 1 vial
K 9652VP	Dilution buffer, ready-to-use	2 x 100 ml
K 9652TMB	TMB substrate (Tetramethylbenzidine), ready-to-use	1 x 15 ml
K 9652AC	ELISA stop solution, ready to use	1 x 7 ml

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Distilled or deionized water
- Precision pipettes calibrated to deliver 10-1000 μ l and disposable tips
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Absorbent paper
- Vortex-Mixer
- Horizontal mixer
- Microplate reader 450 nm

6. PREPARATION AND STORAGE OF REAGENTS

- The **ELISA wash buffer concentrate** should be diluted with aqua dest. **1:10** before use (add 900 ml aqua dest. to 100 ml ELISA wash buffer concentrate). Crystals may be formed due to high salt concentration. The crystals have to be dissolved **before dilution of the buffer concentrate** using a water bath (37°C). The buffer concentrates are stable at 2-8°C up to the expiry date stated on the label. Diluted solutions could be stored at 2-8°C for 1 month.
- The **Controls** have to be reconstituted with 250 μ l aqua dest. **Reconstituted controls are not stable and could not be stored.** Allow the vial to stand for minimum 10 minutes and then mix thoroughly by gentle conversion to insure complete reconstitution.
- The **Conjugate** (POD labelled antibody) vial has to be diluted 1:100 with wash buffer (100 μ l Conjugate + 10 ml wash buffer) and mixed well. The antibody is stable at 2 -4 °C up to the expiry date stated on the label. **Diluted antibody solution is not stable and could not be stored.**

7. PRECAUTIONS

- For in vitro diagnostic 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, plasma

Collection and storage of serum: Collect sufficient blood (at least 1 ml) by venipuncture into a tube or a plastic syringe, avoid hemolysis, centrifuge for 15 minutes at 1,000 x g and 4°C and collect the serum.

Serum samples have to be diluted 1:200 before performing the assay.

Add 5 μ l serum to 995 μ l dilution buffer, mix well. (1:200)

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

Wash the precoated microtiter plate 5 x with 250 μ l ELISA wash buffer. Carry out the tests in duplicate.

1. Add **100 μ l** samples and controls.
2. Incubate **over night (16-20 h)**, on a horizontal mixer, at 2 – 8 °C.
3. Decant the content of the plate and wash the wells **5 x with 250 μ l** ELISA wash buffer.
4. Add **100 μ l** prediluted Peroxidase-labelled antibody.
5. Incubate for **1 hour**, shaking on a horizontal mixer, at room temperature.
6. Decant the content of the plate and wash the wells **5 x with 250 μ l** ELISA wash buffer.
7. Add **100 μ l** TMB substrate solution into each well.
8. Incubate for **5 - 10 minutes** at room temperature in the dark.
9. Add **50 μ l** stop solution into each well and mix shortly.

10. Determine absorption with an ELISA reader at **450 nm** against 620 nm as reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the measurement range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as reference.

10. RESULTS

The results are to determine by cut off. The cut off is twice the OD of the negative control.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

The precision (intra-assay variation) of the Immundiagnostik anti-TNF α antibody ELISA test was calculated from 10 replicate determinations on one positive sample.

Intra-Assay CV n= 10

Sample	Mean value OD	Intra-Assay CV [%]
1	0.340	6

12. 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.
- 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 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.

06/10/2005 06.10.2005 _antiTNFa.DOC