Manual

*IDK*monitor® Etanercept drug level ELISA

For the in vitro determination of free etanercept concentration (e. g. ENBREL®) in EDTA plasma and serum

Valid from 2019-02-07

REF **KR9646**

Σ 96

+8 °C / +2 °C

RUO

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1. INTENDED USE

The Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of free therapeutic TNFα antibodies Etanercept (e. g. ENBREL®) in EDTA plasma and serum. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

The *IDK*monitor® Etanercept drug level ELISA for the determination of the drug level of Etanercept (e. g. ENBREL®) measures quantitatively free Etanercept in EDTA plasma and serum.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
KR9646	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
KR9646	CONJ	Conjugate concentrate, peroxidase-labelled	1 x 200 µl
KR9646	STD	Calibrators, lyophilised (0; 4.15; 8.3; 25; 75; 225 ng/ml)	2 x 6 vials
KR9646	CTRL 1	Control, lyophilised (see specification for range)	2 x 1 vial
KR9646	CTRL 2	Control, lyophilised (see specification for range)	2 x 1 vial
KR0004.100	SAMPLEBUF	Sample dilution buffer, ready-to-use	2 x 100 ml
KR0002.15	SUB	Substrate (tetramethylbenzidin), ready-to-use	1 x 15 ml
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate

- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION 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 run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. Before use, the STD and CTRL have to be reconstituted with **500 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. **Standards and controls** (reconstituted STD and CTRL) **can be stored at -20 °C for 3 months. Avoid repeated thawing and freezing.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in **wash buffer** (100 µl CONJ + 10 ml wash buffer). The **CONJ** is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **is not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

EDTA plasma and serum

Sample storage

Freshly collected EDTA plasma or serum can be stored for 7 days at room temperature (15–30 °C) [6]. The samples can be stored at -20 °C for 6 months.

Diluted EDTA plasma or serum samples can be stored for 7 days at 2–8 °C and at least for 4 weeks at -20 °C. Repeated freezing and thawing is to be avoided.

Sample dilution

EDTA plasma or serum samples must be diluted **1:50** in sample dilution buffer (SAMPLEBUF) before performing the assay, e. g.

10 µl sample + **490 µl** SAMPLEBUF, mix well.

For testing in duplicates, pipet 2 x 100 µl of each prepared sample per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed to determine the quantity of free Etanercept (therapeutic antibody agains TNFα, e. g. ENBREL®) in EDTA plasma or serum samples. In a first incubation step, the free Etanercept from the sample is bound to the specific monoclonal anti-Etanercept antibody coated on the plate. To remove all unbound substances, a washing step is carried out. In a further incubation step, peroxidase-labelled antibody is added. Tetramethylbenzidine (TMB) is used as a substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The colour changes from blue to yellow. The intensity of the yellow colour is directly proportional to the concentration of free Etanercept in the sample. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. Free Etanercept, present in the samples, is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each **100 µl standards/controls/diluted samples** into the respective wells.
3.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add **100 µl conjugate** (diluted CONJ) into each well.
6.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add **100 µl substrate** (SUB) into each well.
9.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
10.	Add **100 µl stop solution** (STOP) into each well and mix well.
11.	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.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. 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 standard must be specified with a value less than 1 (e. g. 0.001).

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 linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

EDTA-plasma and serum samples

The obtained results have to be multiplied by the **dilution factor of 50** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

LoB × *sample dilution factor to be used*

LoB see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

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

Reference range

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n = 40

The repeatability was assessed with 2 serum-samples under **constant** parameters (same operator, measurement system, day and kit lot).

Sample	Mean value [µg/ml]	CV [%]
1	1.66	7.4
2	5.54	9.2

Reproducibility (Inter-Assay); n = 14

The reproducibility was assessed with 2 serum-samples under **varying** parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [µg/ml]	CV [%]
1	2.26	5.5
2	6.68	9.7

Analytical sensitivity

The following value has been estimated based on the concentrations of the standards without considering possibly used sample dilution factors.

Limit of blank, LoB 1.438 ng/ml

12. PRECAUTIONS

- All reagents in the kit package are for research use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin® as bactericides. Sodium azide and ProClin® are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- 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 up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories should be followed.
- *IDK*monitor® is a trademark of Immundiagnostik AG.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure,

which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.

- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

1. Afif W, Loftus EJ, Faubion W, et al. Clinical utility of measuring Etanercept and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. *American Journal of Gastroenterology*. 2010;**105**(5):1133–9.
2. Beglinger C, Binek J, Braegger C, Michetti P, Rogler G, Sauter B, Seibold F, Straumann A (2008) Monotherapie versus Kombinationstherapie mit Immunmodulatoren. *TMI* **1**:32-34
3. Bender NK, Heilig CE, Dröll B, Wohlgemuth J, Armbruster FP, Heilig B (2007) Immunogenicity, efficacy and adverse events of adalimumab in RA patients. *Rheumatol Int*. Jan;**27**(3):269-74
4. Bendtzen K, Geborek P, Svenson M, Larsson L, Kapetanovic MC, Saxne T (2006) Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor alpha inhibitor Etanercept. *Arthritis Rheum*. Dec;**54**(12):3782-9
5. Bradley JR. (2008) TNF-mediated inflammatory disease. *Journal of Pathology*. **214**:149–160.
6. St Clair EW, Wagner CL, Fasanmade AA, Wang B, Schaible T, Kavanaugh A, Keystone EC (2002) The relationship of serum Etanercept concentrations to clinical improvement in rheumatoid arthritis: results from ATTRACT, a multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum*. Jun;**46**(6):1451-9
7. Chang JT, Lichtenstein GR (2006) Drug insight: antagonists of tumor-necrosis factor-alpha in the treatment of inflammatory bowel disease. *Nat Clin Pract Gastroenterol Hepatol*. Apr;**3**(4):220-8. Review
8. Colombel JF, Loftus EV Jr, Tremaine WJ, Egan LJ, Harmsen WS, Schleck CD, Zinsmeister AR, Sandborn WJ (2004) The safety profile of Etanercept in patients with Crohn's disease: the Mayo clinic experience in 500 patients. *Gastroenterology*. Jan;**126**(1):19-31
9. Cominelli F (2004) Cytokine-based therapies for Crohn's disease--new paradigms. *N Engl J Med*. Nov **11**;351(20):2045-8

10. Cornillie F, Shealy D, D'Haens G, Geboes K, Van Assche G, Ceuppens J, Wagner C, Schaible T, Plevy SE, Targan SR, Rutgeerts P (2001) Etanercept induces potent anti-inflammatory and local immunomodulatory activity but no systemic immune suppression in patients with Crohn's disease. *Aliment Pharmacol Ther.* Apr;**15**(4):463-73

11. Maser EA, Villela R, Silverberg MS, Greenberg GR (2006) Association of trough serum Etanercept to clinical outcome after scheduled maintenance treatment for Crohn's disease. *Clin Gastroenterol Hepatol*. Oct;**4**(10):1248-54

12. Rutgeerts P, Van Assche G, Vermeire S (2004) Optimizing anti-TNF treatment in inflammatory bowel disease. *Gastroenterology*. May;**126**(6):1593-610. Review

13. Vande Casteele N, Gils A. Pharmacokinetics of anti-TNF monoclonal antibodies in inflammatory bowel disease: Adding value to current practice. *Journal of clinical pharmacology*. 2015;**55 Suppl 3**:S39–50. doi:10.1002/jcph.374.

14. Perry, M., Bewshea, C., Brown, R., So, K., Ahmad, T., & McDonald, T. (2015). *Etanercept and adalimumab are stable in whole blood clotted samples for seven days at room temperature. Annals of Clinical Biochemistry*. Epub ahead of print.

Used symbols:


Temperature limitation

Catalogue Number

For research use only

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet