**Manual**

# *IDK*monitor® Adalimumab free ADA ELISA

***For the determination of free human antibodies against adalimumab (e. g. HUMIRA®) in EDTA plasma and serum***

Valid from 2019-02-15

REF **KR9652**

Σ 96

+8 °C
+2 °C

RUO

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## 1. INTENDED USE

This enzyme-linked immunosorbent assay (ELISA) kit is intended for the determination of free anti-drug antibodies (ADA) against the therapeutic TNFα antibody adalimumab (e. g. HUMIRA®) in EDTA plasma and serum. For research use only. Not for use in diagnostic procedures.

## 2. INTRODUCTION

The *IDK*monitor® Adalimumab free ADA ELISA for the detection of antibodies against Adalimumab (e.g. HUMIRA®) measures free anti-Adalimumab antibodies.

## 3. MATERIAL SUPPLIED

| Cat. No. | Label | Kit components | Quantity |
|---|---|---|---|
| KR9652 | PLATE | Holder with strips, precoated with $(F(ab)_2)$ | 12 x 8 wells |
| KR0001.C.100 | WASHBUF | Wash buffer concentrate 10x | 1 x 100 ml |
| KR9652 | CONJ | Antibody concentrate, (therapy antibody, peroxidase-labelled) | 1 x 200 µl |
| KR9652 | CTRL NEG | Negative control, lyophilised | 4 x 1 vial |
| KR9652 | CTRL POS | Positive control, lyophilised | 4 x 1 vial |
| KR9652 | CTRL CUT-OFF | Cut-off control, lyophilised | 4 x 1 vial |
| KR0004.100 | SAMPLEBUF | Sample buffer, ready-to-use | 1 x 30 ml |
| KR0002.15 | SUB | Substrate (tetramethylbenzidine), 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
- Absorbent paper
- 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. PREPARATION AND STORAGE OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the 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 controls (CTRL NEG, CTRL POS and CTRL CUT-OFF)** are stable at **2–8 °C** until the expiry date stated on the label. Before use, the CTRL NEG, CTRL POS and CTRL CUT-OFF have to be reconstituted with each **300 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. **Controls** (reconstituted CTRL NEG, CTRL POS and CTRL CUT-OFF) **are not stable and cannot be stored.**
- **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**

EDTA plasma or serum samples must be diluted **1:10** before performing the assay,

e. g. **25 µl** sample + **225 µl** SAMPLEBUF (sample buffer), mix well.

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

**Sample storage**

Undiluted samples can be stored for 1 month at -20 °C and for 7 days at 2–8 °C or room temperature.

Diluted samples are not stable and cannot be stored.

## 7. ASSAY PROCEDURE

### *Principle of the test*

This enzyme immunoassay is a sandwich assay for the determination of free antibodies against adalimumab (e. g. HUMIRA®). In a first incubation step, the free anti-therapeutic antibodies from the sample are bound to the adalimumab $F(ab)_2$ fragments coated on the plate. To remove all unbound substances, a washing step is carried out. In a further incubation step, peroxidase-labelled adalimumab 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 colour converts to yellow. The absorbance of the colour compound is determined photometrically. The intensity of the colour is directly proportional to the amount of bound ADAs (here: anti-adalimumab antibodies) from the sample. The results are evaluated by a cut-off control.

### *Test procedure*

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

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 the 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. | Wash the microtiter strips **5 x with 250 µl wash buffer before use**. After the final washing step, the inverted microtiter strips should be tapped on absorbent paper. |
| 2. | Add **100 µl of CTRL NEG, CTRL POS, CTRL CUT-OFF** (controls), and **diluted samples** in the wells of the microtiter plate. |
| 3. | Seal the stripes with foil and incubate **over night (16–20 h)**, on a horizontal mixer, at 2–8 °C.* |
| 4. | Aspirate the content of the plate and wash each well **5 x with 250 µl wash buffer**. After the final washing step, the inverted microtiter should be firmly tapped on absorbent paper. |
| 5. | Add **100 µl conjugate** into each well. |
| 6. | Seal the stripes with foil and incubate for **1 hour** shaking on a horizontal mixer at room temperature (15–30 °C). |
| 7. | Aspirate the content of the plate and wash each well **5 x with 250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper. |
| 8. | Add **100 µl of TMB substrate** solution into each well. |
| 9. | Incubate for **10–20 minutes** at room temperature in the dark. ** |
| 10. | Add **100 µl stop solution** into each well and mix shortly. |
| 11. | Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. |

* The above incubation step at 2–8 °C on a horizontal mixer is recommended by the producer. If there is no possibility to incubate at 2–8 °C, while shaking, we recommend to incubate at 2–8 °C without any shaking.

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

## 8. RESULTS

The analysis of the results is done using the cut-off control. Samples with a higher optical density (OD) as the OD of the cut-off control are positive. Samples with an OD lower than the OD of the cut-off control are negative.

**Cut-off = 10 AU/ml = OD of cut-off control**

For the calculation of the sample concentrations, linear regression using a linear or-

dinate and abscissa is recommended.

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.

**Sample calculation for a positive sample**

| | |
|---|---|
| average OD of the sample | 0.735 |
| average OD of cut-off control | 0.065 = 10 AU/ml |
| Concentration of the sample | $\frac{0.735 \times 10\ \text{AU/ml}}{0.065} = 113\ \text{AU/ml}$ |

## 9. LIMITATIONS

The lower limit of the measurement range is the LoB.

LoB see chapter "Performance Characteristics".

Samples with concentrations lower than the measurement range cannot be clearly quantified.

## 10. QUALITY CONTROL

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

Provided 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 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=30**

The repeatability was assessed with 3 serum-samples under constant parameters (same operator, measurement system, day and kit lot).

| Sample | Mean value [AU/ml] | CV [%] |
|---|---|---|
| 1 | 87.56 | 2.0 |
| 2 | 40.81 | 2.4 |
| 3 | 45.87 | 2.1 |

**Reproducibility (Inter-Assay); n=18**

The reproducibility was assessed with 3 serum samples under varying parameters (different operators, measurement systems, days and kit lots).

| Sample | Mean value [AU/ml] | CV [%] |
|---|---|---|
| 1 | 87.29 | 5.2 |
| 2 | 47.83 | 8.3 |
| 3 | 79.05 | 7.9 |

### *Analytical sensitivity*

The following values have been estimated without considering possibly used sample dilution factors:

Limit of blank, LoB 1.615 AU/ml

## 12. PRECAUTIONS

- For research use only.
- Control samples should be analyzed with each run.
- 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 out 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 to assemble wells of different microtiter plates for analysis, even if they are of the same batch as wells from already opened microtiter plates are exposed to different conditions as sealed ones.
- 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 the enclosed manual.

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

- Quality control guidelines 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 wrong use.
- Warranty claims and complaints in respect of 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

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5. N. K. Bender, C. E. Heilig, B. Dröll, J. Wohlgemuth, F. P. Armbruster, and B. Heilig, "Immunogenicity, efficacy and adverse events of adalimumab in RA patients.," *Rheumatology international*, vol. **27**, no. 3, pp. 269–74, Jan. 2007

***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