Manual

*IDK*monitor® Adalimumab total ADA ELISA

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

Valid from 2022-03-31

REF **KR9651** Σ 96

REF **KR9651.20** Σ 20 x 96

+8 °C / +2 °C

RUO

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

Table of Contents

1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the determination of human antibodies against TNFα blocker adalimumab (e. g. HUMIRA®) in the presence of adalimumab in EDTA plasma and serum.

For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

The *IDK*monitor® Adalimumab total ADA ELISA for the detection of total antibodies against adalimumab (e. g. HUMIRA®) measures free and bound antibodies against adalimumab. This assay allows a reliable determination of ADA even in the presence of adalimumab.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity for cat. no.	
			KR9651	KR9651.20
KR9651	PLATE	Microtiter plate, pre-coated	12 x 8 wells	20 x 12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10x	1 x 100 ml	15 x 100 ml
KR9651	TRACER	Tracer concentrate, biotinylated	1 x 600 µl	20 x 600 µl
KR9651	CONJ	Conjugate concentrate, peroxidase-labelled	1 x 600 µl	20 x 600 µl
KR9651	CTRL CUT OFF	Cut-off control, lyophilised	4 x 1 vial	30 x 1 vial
KR9651	CTRL NEG	Negative control, lyophilised (see specification data sheet for range)	4 x 1 vial	30 x 1 vial
KR9651	CTRL POS	Positive control, lyophilised (see specification data sheet for range)	4 x 1 vial	30 x 1 vial

Cat. No.	Label	Kit components	Quantity for cat. no.	
			KR9651	KR9651.20
KR9651	ASYBUF	Assay buffer, ready-to-use	2 x 15 ml	25 x 15 ml
KR9651	ABBUF	Antibody dilution buffer, ready-to-use	1 x 10 ml	20 x 10 ml
KR0002.15	SUB	Substrate (tetramethyl-benzidine), ready-to-use	1 x 15 ml	20 x 15 ml
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml	20 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*
- Precision pipettes and pipette tips for single use with variable volumes from 10-1000 µl
- 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 the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the amount necessary for the particular 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. Due to the high salt concentration, crystals can occur in the concentrate. The crystals must be redissolved at room temperature or in a water bath at 37 °C before dilution of the buffer solutions. 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 container at **2–8 °C for 1 month**.
- **Preparation of controls** see chapter 6 and 7.
- **Preparation of the conjugate and tracer: Tracer concentrate (TRACER) and conjugate concentrate (CONJ) are diluted 1:12 in antibody dilution buffer (ABBUF), a few minutes before use.** Prepare ABBUF in a reaction vessel, then add TRACER and CONJ (e. g. 3000 µl antibody dilution buffer + 300 µl tracer + 300 µl conjugate). TRACER and CONJ are stable at **2–8 °C** until expiry date stated on the label. **Tracer** (1:12 diluted TRACER) **and conjugate** (1:12 diluted CONJ) **are not stable and cannot be stored**.
- All other test reagents are ready-to-use and stable until the expiry date stated on the label, when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Storage of samples

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

Diluted samples are **not stable** and **cannot be stored. Sample dilution** is carried out **simultaneously with the preparation of controls** immediately before starting the test (see chapter 7, section "Test procedure").

7. ASSAY PROCEDURE

Test principle

This ELISA is designed for the quantitative determination of antibodies against TNFα blocker adalimumab (e. g. HUMIRA®).

During sample preparation, the anti-drug antibodies (ADA) are separated from the therapeutic antibody in order to acquire free ADA. By adding the conjugate (peroxidase labelled therapeutic antibody) and the tracer (biotinylated therapeutic anti-

body), the unmarked therapeutic antibodies are replaced and the marked antibodies can form a complex with the ADA. This complex binds via biotin to the streptavidin coated microtiter plate. It is detected via the peroxidase conjugate with the peroxidase converting the substrate TMB to a blue product. The enzymatic reaction is stopped by adding an acidic solution. The samples convert from blue to yellow.

The colour change should be measured in a photometer at 450 nm. The interpretation is made using the cut-off control.

Test procedure

Attention: The prepared sample must **not be stored** and **must be analysed immediately afterwards** in the test.

Sample Preparation

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

1.	Pipette **25 µl sample** into each reaction tube and mix by adding **225 µl assay buffer** (ASYBUF), vortex (sample dilution 1:10). **Attention**: The addition of the assay buffer should be performed as soon as possible for all samples, as this step serves to cleave the ADAs from the therapeutic antibodies. Reconstitute the **controls** with **500 µl assay buffer** and vortex. **Attention**: This should be done **simultaneously with the sample dilution** to ensure equal treatment of controls and samples.
2.	Incubate controls and diluted samples in reaction tubes for **20 min** while **shaking** on a horizontal shaker at room temperature (15–30 °C). **Caution: Incubation time begins upon addition of assay buffer**.
3.	At the end of the incubation period, transfer **250 µl of each control** into a reaction vessel.
4.	Add **60 µl tracer/conjugate/antibody dilution buffer solution** (see chapter 5 "Storage and Preparation of Reagents") to **250 µl** control/diluted sample**.** Vortex and incubate for **1 hour** while **shaking*** at room temperature (15–30 °C).

* We recommend shaking the controls and reaction tubes at 550 rpm with an orbit of 2 mm.

The samples are now ready for testing

Test execution

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

Take as many **microtiter strips** as needed from 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.

5.	**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.
6.	Pipet **100 µl preincubated controls/samples** into the respective wells.
7.	Cover the strips and incubate for **1.5 hours** while **shaking*** on a horizontal shaker at room temperature (15–30 °C).
8.	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.
9.	Add **100 µl substrate** (SUB) into each well.
10.	Incubate for **10–20 minutes**** at room temperature (15–30 °C) in the **dark**.
11.	Add **100 µl stop solution** (STOP) into each well and mix shortly by using the shake function of the microtiter plate reader.
12.	Determine the **absorption** immediately with the microtiter plate reader at **450 nm** against 620 nm (or 690 nm) as reference wavelength.

* 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

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

Samples which have a higher average optical density (OD) than the cut-off control are positive.

For the evaluation of the test we recommend linear regression with linear ordinate and abscissa for optical density and concentration.

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 manual control of the paired values should be made.

Sample calculation for a positive sample

Average OD of sample 0.735

Average OD of cut-off control 0.085 = 10 AU/ml

Concentration sample $\frac{0.735 \times 10 \text{ AU/ml}}{0.085} = 86.47 \text{ AU/ml}$

9. LIMITATIONS

Measurement range

The lower limit of the measurement range is the LoQ.

LoQ see chapter "Performance Characteristics".

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

Biotin interference

Samples containing a biotin concentration of < 100 ng/ml show a change of the results of ≤ 25 %. Higher concentrations of biotin can lead to falsely low results. Persons taking > 5 mg biotin per day should wait at least 24 hours after intake of biotin to have their samples collected. Results of persons taking biotin supplements or receiving a high-dose biotin therapy should generally be interpreted along with the total clinical picture.

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 = 23

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

Sample	Mean value [AU/ml]	CV [%]
1	59.91	3.5
2	290.92	5.9

Reproducibility (Inter-Assay); n = 14

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

Sample	Mean value [AU/ml]	CV [%]
1	17.40	7.1
2	48.11	5.2
3	213.46	5.1

Analytical sensitivity

The following value has been estimated without considering possibly used sample dilution factors

Limit of blank, LoB	2.643 AU/ml
Limit of detection, LoD	5.067 AU/ml
Limit of quantitation, LoQ	10 AU/ml

The specified accuracy goal for the LoQ was 20 % CV.

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 or ProClin are hazardous to health and the environment. Substrates for enzymatic colour reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- The 10x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact.

  Warning: Causes serious eye irritation. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical Advice/attention.
- 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.
- *IDKmonitor*® 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 EV, Jr., Faubion WA, Kane SV, Bruining DH, Hanson KA, Sandborn WJ (2010). Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. *Am J Gastroenterol* **105**(5): 1133-1139.
2. Kopylov U, Mazor Y, Yavzori M, Fudim E, Katz L, Coscas D, Picard O, Chowers Y, Eliakim R, Ben-Horin S (2012). Clinical utility of antihuman lambda chain-based

enzyme-linked immunosorbent assay (ELISA) versus double antigen ELISA for the detection of anti-infliximab antibodies. *Inflamm Bowel Dis* **18**(9): 1628-1633.

3. Tak PP (2012). A personalized medicine approach to biological treatment of rheumatoid arthritis: a preliminary treatment algorithm. *Rheumatology* **51**(4): 600-609.
4. Ordas I, Mould DR, Feagan BG, Sandborn WJ (2012) Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. *Clin Pharmacol Ther* **91**(4): 635-646.
5. Bender NK, Heilig CE, Droll B, Wohlgemuth J, Armbruster FP, Heilig B (2007). Immunogenicity, efficacy and adverse events of adalimumab in RA patients. *Rheumatol Int* **27**(3): 269-274.

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


Irritant