Manual

*IDK*monitor® Golimumab free ADA ELISA

For the determination of free human antibodies against golimumab (e. g. Simponi®) in EDTA plasma and serum

Valid from 2019-08-7

REF **KR9649**

Σ 96

+2 °C +8 °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 golimumab (e. g. Simponi®) in EDTA plasma and serum. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Chronic inflammatory diseases like Crohn's disease, ulcerative colitis, rheumatoid arthritis or psoriasis are often treated with anti-TNFα antibodies which target directly the underlying inflammatory processes.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR9649	PLATE	Microtiter plate, pre-coated with $(F(ab)_2)$	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10x	1 x 100 ml
KR9649	CONJ	Conjugate concentrate (therapy antibody), peroxidase-labelled	1 x 200 µl
KR9649	CTRL NEG	Negative control, lyophilised (see specification for range)	4 x 1 vial
KR9649	CTRL POS	Positive control, lyophilised (see specification for range)	4 x 1 vial
KR9649	CTRL CUT-OFF	Cut-off control, lyophilised	4 x 1 vial
KR9649	SAMPLEBUF	Sample dilution 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
- 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 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. **Reconstitution** details are given in the **specification data sheet**. **Controls** (reconstituted CTRL CUT-OFF, CTRL POS and CTRL NEG) **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. SPECIMEN COLLECTION AND PREPARATION

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 diluted sample.

Sample storage

Freshly collected EDTA plasma or serum can be stored for one day at room temperature (15–30 °C) or for longer storage at -20 °C.

7. ASSAY PROCEDURE

Principle of the test

This enzyme immunoassay is a sandwich assay for the determination of free antibodies against golimumab (e. g. Simponi®). In a first incubation step, the free anti-therapeutic antibodies from the sample are bound to the golimumab $F(ab)_2$ fragments coated on the plate. To remove all unbound substances, a washing step is carried out. In a further incubation step, peroxidase-labelled golimumab 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-golimumab 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.

Mark the positions of 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 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** at 550 rpm with an orbit of 2 mm.
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** at 550 rpm with an orbit of 2 mm.
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 minutes*** at room temperature (15–30 °C) in the **dark**.
10.	Add **100 µl stop solution** (STOP) and mix well.
11.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. 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 colour change is temperature sensitive. We recommend observing the colour change and stopping 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 ordinate 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 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 AG recommends the use of external controls for internal quality control, if possible.

Provided 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 = 20

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	80.47	2.6
2	40.45	3.6
3	20.65	6.2

Reproducibility (Inter-Assay); n = 22

The reproducibility was assessed with 4 serum-samples under **varying** parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [AU/ml]	CV [%]
1	91.15	8.1
2	48.00	9.4
3	25.17	8.9
4	13.08	10.2

Analytical sensitivity

The following values have been estimated based on the concentrations of the calibration curve without considering possibly used sample dilution factors:

Limit of blank, LoB 7.66 AU/ml

12. PRECAUTIONS

- 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 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.
- 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 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

1. Ordás, Ingrid, Diane R Mould, Brian G Feagan, and William J Sandborn. 2012. "Anti-TNF Monoclonal Antibodies in Inflammatory Bowel Disease: Pharmacokinetics-Based Dosing Paradigms." *Clinical Pharmacology and Therapeutics* **91** (4). Nature Publishing Group: 635–46. doi:10.1038/clpt.2011.328.
2. Xu, Zhenhua, Thuy Vu, Howard Lee, Chuanpu Hu, Jie Ling, Hong Yan, Daniel Baker, et al. 2009. "Population Pharmacokinetics of Golimumab, an Anti-Tumor Necrosis Factor-Alpha Human Monoclonal Antibody, in Patients with Psoriatic Arthritis." *Journal of Clinical Pharmacology* **49** (9): 1056–70. doi:10.1177/0091270009339192.
3. Vincent, Fabien B, Eric F Morand, Kim Murphy, Fabienne Mackay, Xavier Mariette, and Christian Marcelli. 2013. "Antidrug Antibodies (ADAb) to Tumour Necrosis Factor (TNF)-Specific Neutralising Agents in Chronic Inflammatory Diseases: A Real Issue, a Clinical Perspective." *Annals of the Rheumatic Diseases* **72** (2): 165–78. doi:10.1136/annrheumdis-2012-202545.

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

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