Manual

*IDK*monitor® Golimumab drug level ELISA

For the determination of free golimumab concentration (e. g. SIMPONI®) in EDTA plasma and serum

Valid from 2019-01-01

REF **KR9656**

Σ 96

+2°C +8°C

RUO

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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of free golimumab (therapeutic antibody against TNFα, 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 increasingly being treated with antibodies against TNFa, which target directly the underlying inflammatory processes.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
KR9656	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
KR9656	CONJ	Conjugate concentrate, 100 x, peroxidase-labelled	1 x 200 µl
KR9656	STD	Standards, ready-to-use (0; 4.15; 8.3; 25; 75; 225 ng/ml)	1 x 6 vials
KR9656	CTRL1	Control, ready-to-use (see specification for range)	1 x 1 vial
KR9656	CTRL2	Control, ready-to-use (see specification for range)	1 x 1 vial
KR0004.100	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 ml
KR0002.15	SUB	Substrate, 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 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**.
- **Preparation of the conjugate:** The **conjugate concentrate (CONJ)** must be diluted **1:101** in wash buffer (e. g. 100 µl CONJ + 10 ml wash buffer). The CONJ is stable at **2–8 °C** until 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

Storage

Freshly collected EDTA plasma or serum can be stored for 1 day at room temperature (15–30 °C). Long term storage is recommended at -20 °C for up to 6 months.

EDTA plasma and serum

EDTA plasma or serum samples must be diluted **1:100** before performing the assay, e.g. **10 µl** sample + **990 µl** dilution buffer (SAMPLEBUF), mix well.

For testing in duplicates, pipette **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 golimumab (therapeutic antibody against TNFα) in EDTA plasma or serum samples. In a first incubation step, the free golimumab from the sample is bound to the specific monoclonal anti-golimumab 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 golimumab 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 standard. The concentrations of free golimumab in the samples are 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.	Add each **100 µl standards/controls/diluted samples** into the respective wells.
2.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
3.	Discard the contents 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.
4.	Add **100 µl conjugate** (diluted CONJ) into each well.
5.	Cover the strips and incubate for **1 hour** at room temperature (15-30 °C) on a **horizontal shaker***.
6.	Discard the contents 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.
7.	Add **100 µl substrate** (SUB) into each well.
8.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
9.	Add **100 µl stop solution** (STOP) into each well, mix.
10.	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 calibrator 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 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.

EDTA-plasma and serum samples

The obtained results have to be multiplied with the **dilution factor of 100** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used to get the real concentration.

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=20

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	13.75	5.5
2	6.40	5.9

Reproducibility (Inter-Assay); n=12

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

Sample	Mean value [µg/ml]	CV [%]
1	6.25	9.0
2	14.78	10.3
3	7.84	4.5
4	2.24	6.2

Analytical sensitivity

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

Limit of blank, LoB 3.647 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. 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.
2. Xu, Z. et al. Population pharmacokinetics of golimumab, an anti-tumor necrosis factor-alpha human monoclonal antibody, in patients with psoriatic arthritis. *J. Clin. Pharmacol.* **49**, 1056–70 (2009).
3. Vincent, F. B. et al. Antidrug antibodies (ADAb) to tumour necrosis factor (TNF)-specific neutralising agents in chronic inflammatory diseases: a real issue, a clinical perspective. *Ann. Rheum. Dis*. **72**, 165–78 (2013).

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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