Manual

*IDK*monitor® Certolizumab drug level ELISA

For the determination of free Certolizumab concentration (e. g. Cimzia®) in serum

Valid from 2022-02-22

REF **KR9662**

Σ 96

+8 °C / +2 °C

RUO

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1. INTENDED USE

*IDK*monitor® Certolizumab drug level ELISA is an enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of the free anti-TNFα therapy antibody Certolizumab (e.g. Cimzia®) in human serum. This assay is intended to be used by professional users in a laboratory environment. Not for use in diagnostic procedures. For research use only.

2. INTRODUCTION

Certolizumab is the Fab' fragment of a recombinant humanized antibody conjugated to polyethylene glycol (PEG). Therefore, this is also called certolizumab pegol.

Certolizumab pegol (Cimzia®) is a TNF-alpha inhibitor used for the treatment of chronic inflammatory diseases, such as Crohn's disease [1] and rheumatoid arthritis [2] [3]. Tumor necrosis factor alpha (TNFα) is one of the proinflammatory cytokines that promote and maintain inflammatory responses. Therefore, the treatment of chronic inflammatory diseases is increasingly performed with antibodies against TNFα, which directly interfere with the underlying inflammatory response. The efficacy of the therapy usually correlates with the amount of therapy antibody detectable in the 's serum shortly before the next drug administration, the so-called trough level. Various factors influence the level of the trough. These include the dose and frequency of anti-TNFα treatment, disease activity, individual differences in pharmacokinetics, and the presence of anti-drug antibodies (ADA) [4,5].

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
KR9662	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
KR9662	CONJ	Conjugate, peroxidase-labeled, ready-to-use	1 x 12.5 ml
KR9662	STD	Calibrators, ready-to-use (see specification for concentrations)	1 x 6 vials
KR9662	CTRL 1	Control 1, ready-to-use (see specification for concentrations)	1 x 1 vial
KR9662	CTRL 2	Control 2, ready-to-use (see specification for concentrations)	1 x 1 vial
KR9662	SAMPLEBUF	Dilution buffer, ready-to-use	1 x 80 ml

Cat. No.	Label	Kit Components	Quantity
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

- Ultra pure water*
- Calibrated precision pipettors and 10–1 000 µl tips
- Absorbent paper
- Thermo shaker (25 °C)
- Multi-channel pipets or repeater pipets
- Centrifuge
- Vortex
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of Ultra Pure 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 ultra pure water **1:10** before use (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solution. 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 flask at **2–8 °C for one month**.

- All other test reagents are ready-to-use. Test reagents, except microtiter strips (PLATE) – see "Test procedure" in chapter "Assay Procedure" –, are stable until the expiry date (see label of test package) when stored at 2–8 °C.

6. STORAGE AND PREPARATION OF SAMPLES

Serum

Serum samples must be diluted **1:1 000** with dilution buffer (SAMPLEBUF) before performing the assay, e. g.

- **10 µl** sample + **490 µl** SAMPLEBUF = **1:50** (**dilution I**)
- **20 µl** dilution I + **380 µl** SAMPLEBUF = **1:20** (**dilution II**).
  This results in a **total dilution of 1:1 000** (= diluted SAMPLE).

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

Sample storage

Freshly collected serum can be stored for 7 days at 2–8 °C. At -20 °C, the samples are stable for 6 months.

Diluted serum samples can't be stored.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed to determine the quantity of free certolizumab (therapeutic antibody against TNFα) in serum samples. In a first incubation step, the free certolizumab from the sample is bound TNFα coated on the plate. To remove all unbound substances, a washing step is carried out. In a further incubation step, certolizumab-specific peroxidase-labeled 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 certolizumab in the sample. A dose response curve of the absorbance unit (optical density, OD) vs. concentration is generated, using the values obtained from standard. The concentrations of free certolizumab in the samples are determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (20–30 °C) and mix well.

Take as many microtiter strips as needed from kit. Store unused strips covered with the provided foil (FOL) together with the desiccant bag in the re-closed aluminum packaging at 2–8 °C and use them in the next 11 months.

We recommend to carry out the tests in duplicate.

1.	Add **100 µl of standards**, **controls** or **samples** (diluted SAMPLE) into the respective wells.
2:	Cover the plate tightly and incubate for **1 hour** at 25 °C on a thermo shaker at **550 rpm**.
3.	Discard the contents of each well. Wash each well **5 x** by dispensing **250 µl of wash buffer** into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
4.	Add **100 µl conjugate** (CONJ) into each well.
5.	Cover the plate tightly and incubate for **1 hour** at 25 °C on a thermo shaker at **550 rpm**.
6.	Discard the contents of each well. Wash each well **5 x** by dispensing **250 µl of wash buffer** into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
7.	Add **100 µl substrate** (SUB) into each well.
8.	Incubate for **15–20 min*** at room temperature (20–30 °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.

*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 algorithm can be used to calculate the results.

Point-to-point calculation

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 program used, the duplicate values should be evaluated manually.

Serum samples

The obtained Certolizumab levels of serum samples have to be multiplied with the dilution factor of 1 000. The resulting value has the unit ng/ml. To convert the value to µg/ml, divide it by 1 000.

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

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) must be further diluted and re-assayed. Please consider this greater 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
*300 ng/ml * 1 000 = 300 000 ng/ml = 300 µg/ml*

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

LoQ × sample dilution factor to be used:
*5.1 ng/ml * 1 000 = 5 100 ng/ml = 5.10 µg/ml*

Analytical sensitivity see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik 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=32

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	82.69	6.0
2	43.09	6.1

Reproducibility (Inter-Assay); n=60

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

Sample	Mean value [µg/ml]	CV [%]
1	69.1	11.5
2	21.3	13.2
3	88.6	6.6

Analytical sensitivity

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

Limit of blank, LoB 3.7 ng/ml

Limit of detection, LoD 5.1 ng/ml

Limit of quantitation, LoQ 5.1 ng/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 should 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 the 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. Sandborn WJ, et al., Certolizumab Pegol for the Treatment of Crohn's Disease. N Engl J Med 2007, 357:228-238. DOI: 10.1056/NEJMoa067594
2. Smolen JS, et al. Head-to-head comparison of certolizumab pegol versus adalimumab in rheumatoid arthritis: 2-year efficacy and safety results from the randomised EXXELERATE study. Lancet 2016;388:2763–74.OT
3. Niti Goel & Sue Stephens (2010) Certolizumab Pegol, mAbs, 2:2, 137-147, DOI: 10.4161/mabs.2.2.11271
4. 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 Mar;55 Suppl 3(May 2014):S39-50.
5. Gehin, J.E., Goll, G.L., Warren, D.J. et al. Associations between certolizumab pegol serum levels, anti-drug antibodies and treatment response in s with inflammatory joint diseases: data from the NOR-DMARD study. Arthritis Res Ther 1,256 (2019). https://doi.org/10.1186/s13075-019-2009-5

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

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