Manual

*IDK*monitor® Rituximab drug level ELISA

For the in vitro determination of free Rituximab concentration (e. g. MabThera®, Rituxan®) in EDTA plasma and serum

Valid from 2022-02-09

REF **KR9661**

Σ 96

+8 °C / +2 °C

RUO

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1. INTENDED USE

The Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of free chimeric anti-CD20 therapeutic antibody rituximab (e. g. MabThera®, Rituxan®) in EDTA plasma and serum. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Rituximab is a chimeric monoclonal antibody against the B cell surface antigen CD20. CD20 (also known as human B-lymphocyte-restricted differentiation antigen or Bp35) is a surface antigen on normal and malign pre-B-lymphocytes and mature B-lymphocytes, but it is not expressed on hematopoietic stem cells, pro-B-lymphocytes, plasma cells or cells of other tissues. CD20 supports the B-cell immune response, in particular against T-cell-independent antigenes [6] and maybe works as a calcium channel.

By binding to CD20, rituximab improves i. a. the effect of natural killer (NK) cells which induce cell death in the B-lymphocytes marked with rituximab [7]. Hereby the number of alive B-lymphocytes is clearly reduced.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
KR9661	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
KR9661	CONJ	Conjugate, peroxidase-labelled, ready-to-use	1 x 15 ml
KR9661	STD	Calibrators, ready-to-use (see specification for concentrations)	1 x 6 vials
KR9661	CTRL 1	Control 1, ready-to-use (see specification for range)	1 x 1 vial
KR9661	CTRL 2	Control 2, ready-to-use (see specification for range)	1 x 1 vial
KR9661	SAMPLEBUF	Dilution buffer, ready-to-use	1 x 100 ml
KR0002.15	SUB	Substrate (tetramethylbenzidin), ready-to-use	1 x 15 ml

Cat. No.	Label	Kit Components	Quantity
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 10–1 000 µl single-use tips
- Absorbent paper
- Thermo shaker (25 °C)
- 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 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 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, except microtiter strips (PLATE) - see "Test procedure" in chapter "Assay Procedure". Test reagents are stable until the expiry date (see label of test package) when stored at 2–8 °C.

6. STORAGE AND PREPARATION OF SAMPLES

EDTA plasma and serum

EDTA plasma or serum samples must be diluted **1:2 000** with sample dilution buffer (SAMPLEBUF) before performing the assay, e. g.

- **10 µl** sample + **490 µl** SAMPLEBUF = **1:50** (**dilution I**)
- **10 µl** dilution I + **390 µl** SAMPLEBUF = **1:40** (**dilution II**).
  This results in a **total dilution of 1:2 000** (= diluted SAMPLE).

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

Sample storage

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

Diluted EDTA plasma or serum samples can be stored for 7 days at 2–8 °C.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed to determine the quantity of free Rituximab (therapeutic antibody against CD20) in EDTA plasma or serum samples. In a first incubation step, the free Rituximab from the sample is bound to the specific monoclonal anti-Rituximab 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 Rituximab 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 Rituximab 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 aluminium packaging at 2–8 °C and use them in the next 4 months.

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 **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 **300 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 **300 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 **10–15 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 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 standard 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 duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

EDTA-plasma and serum samples

The obtained Rituximab levels of EDTA plasma and serum samples have to be multiplied by the dilution factor of 2 000. 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 by the dilution factor used.

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
*250 ng/ml * 2 000 = 500 000 ng/ml = 500 µg/ml*

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

LoQ × sample dilution factor to be used:
*2,54 ng/ml * 2 000 = 5 080 ng/ml = 5,080 µg/ml*

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 = 32

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

Sample	Mean value [µg/ml]	CV [%]
1	38.96	2.9
2	210.15	7.2

Reproducibility (Inter-Assay); n = 24

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

Sample	Mean value [µg/ml]	CV [%]
1	38.99	9.4
2	231.42	9.6

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	1.59 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 harmful to health and the environment. Substrates for enzymatic color reactions can also cause skin and/or respiratory irritation. Any contact with the substances should be avoided. Further safety information can be found in the safety data sheet, which you can obtain 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

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3. Singer O, McCune WJ. Update on maintenance therapy for granulomatosis with polyangiitis and microscopic polyangiitis. *Current opinion in rheumatology*. 2017 May;**29**(3):248–53.

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5. Tandan R, Hehir MK, Waheed W, Howard DB. Rituximab treatment of myasthenia gravis: A systematic review. *Muscle & nerve*. 2017 Aug;**56**(2):185–96.
6. Kuijpers TW, Bende RJ, Baars PA, Grummels A, Derks IAM, Dolman KM, et al. CD20 deficiency in humans results in impaired T cell-independent antibody responses. *The Journal of clinical investigation*. 2010 Jan;**120**(1):214–22.
7. Rudnicka D, Oszmiana A, Finch DK, Strickland I, Schofield DJ, Lowe DC, et al. Rituximab causes a polarization of B cells that augments its therapeutic function in NK-cell-mediated antibody-dependent cellular cytotoxicity. *Blood*. 2013 Jun 6;**121**(23):4694–702.

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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