Manual

*IDK*monitor® Ustekinumab total ADA ELISA

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

Valid from 2022-02-17

REF **KR9667**

Σ 96

+8 °C
+2 °C

RUO

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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the determination of human anti-drug antibodies (ADA) against the therapeutic antibody ustekinumab (e. g. STELARA®) in the presence of ustekinumab in EDTA plasma and serum. For *in vitro* diagnostic use only.

2. INTRODUCTION

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

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR9667	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10x	1 x 100 ml
KR9667	TRACER	Tracer concentrate, biotinylated	1 x 600 µl
KR9667	CONJ	Conjugate concentrate, peroxidase-labelled	1 x 600 µl
KR9667	CTRL CUT-OFF	Cut-off control, lyophilised	4 x 1 vial
KR9667	CTRL NEG	Negative control, lyophilised (see specification for range)	4 x 1 vial
KR9667	CTRL POS	Positive control, lyophilised (see specification for range)	4 x 1 vial
KR9667	ASYBUF	Assay buffer, ready-to-use	2 x 15 ml
KR9667	ABBUF	Antibody dilution buffer, ready-to-use	1 x 10 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 10–1 000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- 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

- **Bring all reagents to room temperature (15–30 °C) prior to use.**
- 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.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** should 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. 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 1 month**.
- **Preparation of controls** see chapter 6.
- **Preparation of the conjugate and tracer: A few minutes before use, tracer concentrate (TRACER)** and **conjugate concentrate (CONJ)** have to be diluted **1:12** in **antibody dilution buffer (ABBUF)**: first put the ABBUF, then add successively TRACER and CONJ (e. g. 2500 µl antibody dilution buffer + 250 µl tracer + 250 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. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. PREPARATION OF THE ASSAY

Preparation of samples and controls

1.	Dilute samples **1:10** in **assay buffer** (ASYBUF) by pipetting **25 µl sample** into a reaction tube and adding **225 µl assay buffer**. Mix well. Addition of assay-buffer to all the samples should be performed without pause since this step dissociates the antibody–therapeutic antibody complexes. Reconstitute the **controls** with **250 µl assay buffer** and vortex. Carry out this step simultaneously with sample dilution in order to ensure equal treatment of controls and samples.
2.	Incubate controls and diluted samples in reaction tubes for **20 min** with **shaking** on a horizontal shaker at room temperature. **CAUTION**: incubation time begins **upon addition of assay buffer**.
3.	Add **60 µl tracer/conjugate/antibody dilution buffer solution** (see preparation of reagents) to **250 µl** control/diluted sample. Vortex and incubate for **1 hour** with **shaking** at room temperature (15–30 °C).

7. ASSAY PROCEDURE

Principle of the test

This ELISA serves for the determination of antibodies against TNF therapeutic antibody ustekinumab (e. g. STELARA®). During sample preparation, the anti-drug antibodies (ADA) are separated from the therapeutic antibody in order to acquire free ADA. By adding the peroxidase conjugate (peroxidase labelled therapeutic antibody) and the tracer (biotinylated therapeutic antibody), 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 cutoff 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 (PLATE) 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 the 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.	Pipet **100 µl of preincubated controls/samples** into the wells of the microtiter plate.
3.	Cover the strips and incubate for **1.5 hours shaking*** on a horizontal shaker at room temperature (15–30 °C).
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 substrate** (SUB) into each well.
6.	Incubate for **10–20 min***** at room temperature (15–30 °C) in the **dark**.
7.	Add **100 µl stop solution** (STOP) into each well and mix shortly by using the **shake function**** of the microplate reader.
8.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 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

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.

Immundiagnostik AG recommends linear regression using a linear ordinate and abscissa to calculate the results.

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 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.

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 samples under **constant** parameters (same operator, instrument, day and kit lot).

Sample	Mean value [AU/ml]	CV [%]
1	39.42	3.5
2	22.28	3.3
3	12.04	8.5

Reproducibility (Inter-Assay); n = 10

The reproducibility was assessed with 5 samples under **varying** parameters (different operators, instruments, days and kit lots).

Sample	Mean value [AU/ml]	CV [%]
1	54.03	17.8
2	93.50	15.8
3	59.03	22.1
4	16.49	20.8
5	11.03	19.6

Analytical Sensitivity

Limit of blank, LoB 4.964 AU/m

Limit of detection, LoD 7.388 AU/ml

Limit of quantitation, LoQ 10 AU/ml

The specified accuracy goal for the LoQ was 10 % 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 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 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

- 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 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.
- *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. LITERATURE

1. Scherl EJ, Kumar S, Warren RU. Review of the safety and efficacy of ustekinumab. Therapeutic Advances in Gastroenterology. 2010;3(5):321–8.
2. Chiu HY, Chu TW, Cheng YP, Tsai TF. The association between clinical response to ustekinumab and immunogenicity to ustekinumab and prior adalimumab. PLoS ONE. 2015;10(11):1–10.
3. Barré A, Colombel JF, Ungaro R. Review article: predictors of response to vedolizumab and ustekinumab in inflammatory bowel disease. Aliment Pharmacol Ther. 2018;47(7):896–905.
4. Jauregui-Amezaga A, Somers M, De Schepper H, Macken E. Next generation of biologics for the treatment of Crohn's disease: an evidence-based review on ustekinumab. Clin Exp Gastroenterol. 2017;10:293–301.
5. Vermeire S, Gils A, Accossato P, Lula S, Marren A. Immunogenicity of biologics in inflammatory bowel disease. Therap Adv Gastroenterol. 2018;11:1756283X17750355.

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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