Manual

anti-heTG IgA ELISA

For the determination of anti-epidermal transglutaminase IgA antibodies in serum and plasma

Valid from 2019-03-21

REF **K 9396**

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 IgA anti-epidermal-transglutaminase antibodies in serum and plasma. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Transglutaminases are enzymes that catalyse cross-links between proteins. Eight human, calcium-dependent transglutaminases are known. Transglutaminase 3 (TGM3), also known as transglutaminase E, TG (E) or epidermal transglutaminase, is expressed in the epidermis and involved in the final differentiation of hair follicles and keratinocytes.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR9396	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10x	2 x 100 ml
KR9396	CTRL NEG	Control negative, lyophilised (see specification for concentration)	4 x 1 vial
KR9396	CTRL POS	Control positive, lyophilised (see specification for concentration)	4 x 1 vial
KR9396	CTRL CUT-OFF	Cut-off control, lyophilised (see specification for concentration)	4 x 1 vial
KR9396	CONJ	Conjugate concentrate, peroxidase-labelled (rabbit-anti-IgA antibody)	1 x 200 µl
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, 3000 *g*
- 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**.
- The **lyophilised negative, positive and cut-off controls** (CTRL NEG, CTR 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 data sheet. **Diluted controls 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. STORAGE AND PREPARATION OF SAMPLES

Serum / Plasma

EDTA plasma or serum samples must be diluted **1:250 in washbuffer** before performing the assay, e.g.

10 µl sample + **90 µl** SAMPLEBUF washbuffer), mix well. = dilution I (1:10)

40 µl sample + **960 µl** SAMPLEBUF washbuffer), mix well. = dilution II (1:25)

For analysis, pipet **100 µl** of **dilution II** per well.

Sample storage

Serum and plasma samples are stable at -20 °C for up to 6 months. Repeated freezing and thawing should be avoided.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of anti-epidermal transglutaminase antibodies (IgA anti-heTG). The wells of the microtiter plate are coated with the antigen. In a first incubation step, the anti-heTG antibodies are bound to the coated antigen. To remove all unbound substances, a washing step is carried out. In a further incubation step, a peroxidase-labelled antibody is added. After another washing step, to remove all unbound substances, the solid phase is incubated with the peroxidase substrate, tetramethylbenzidine (TMB). Finally, an acidic stop solution is added to terminate the enzymatic reaction, whereby the colour changes from blue to yellow. The intensity of the yellow colour is directly proportional to the IgA anti-epidermal-transglutaminase antibody concentration. 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***.
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***.
7.	Discard the contents of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
8.	Add **100 µl substrate** (SUB) into each well.
9.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
10.	Add **100 µl stop solution** (STOP) into each well and mix well.
11.	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

Since the sample dilution of 1:250 is already considered in the cut-off control, the dilution factor is 1.

Samples with an optical density higher than the average optical density of the cut-off control are positive.

Cut-off = $OD_{cut\text{-}off\ control}$ = 22 AU/ml

Example

OD_{sample} = 0.685

$OD_{cut\text{-}off\ control}$ = 0.234 = 22 AU/ml

$$\text{Concentration sample} = \frac{0{,}685 * 22\ \text{AU/ml}}{0{,}234} = 64{,}4\ \text{AU/ml}$$

Attention: Calculation is only valid for a sample dilution factor of **1:250**.

9. LIMITATIONS

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

LoB × sample dilution factor to be used

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=64

The repeatability was assessed with one serum sample under constant parameters (same operator, measurement system, day and kit lot).

Sample	Mean value [AU/ml]	CV [%]
1	37.15	5.2

Reproducibility (Inter-Assay); n=24

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

Sample	Mean value [AU/ml]	CV [%]
1	25.48	8.8
2	9.18	14.3

Analytical sensitivity

Limit of blank, LoB 2.874 AU/ml

The evaluation was performed according to the CLSI guideline EP17-A2.

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 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.
- 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.

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