Manual

IDK® anti-hnTG IgA ELISA

For the determination of autoantibodies (IgA) against human transglutaminase 6 in plasma and serum

Valid from 2021-07-12

REF **KR9400**

Σ 96

+8 °C
+2 °C

RUO

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Table of Contents

1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of anti-hnTG IgA ELISA in serum and plasma. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Autoantibodies directed against neuronal transglutaminase (TG6) have been detected in sera from subjects with neurological disorders (e. g. ataxia, neuropathies, cerebral palsy, or stiff-person syndrome).

TG6 autoantibodies may be present in subjects with celiac disease in addition to TG2 autoantibodies, but have also been found in TG2 autoantibody-negative sera and in sera from subjects without enteropathy (i. e. without celiac disease).

This ELISA is for the quantitative detection of antibodies to TG6 in human serum.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR9400	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
KR9400	CONJ	Conjugate, ready-to-use	1 x 15 ml
KR9400	STD	Standards, lyophilised (see specification for concentrations)	4 x 5 vials
KR9400	CTRL1	Control, lyophilised (see specification for range)	4 x 1 vial
KR9400	CTRL2	Control, lyophilised (see specification for range)	4 x 1 vial
KR9400	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 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

- Ultra pure 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 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 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.
- **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 concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** can be used until the expiry date stated on the label when stored at **2–8 °C**. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- The **lyophilised standards (STD)** and **controls (CTRL)** can be used until the expiry date stated on the label when stored at **2–8 °C**. **Reconstitution** details are given in the **specification data sheet**. **Standards and controls** (reconstituted STD and CTRL) **are not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents can be used until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage

Avoid repeated thawing and freezing of samples.

Sample dilution

EDTA plasma or serum samples must be diluted **1:250** in sample dilution bufffer (SAMPLEBUF) before performing the assay, e.g.

- **20 µl** sample + **180 µl** SAMPLEBUF, mix well = **1:10** (**dilution I**)
- **20 µl** dilution I + **480 µl** SAMPLEBUF, mix well = **1:25** (**dilution II**)

100 µl of the **dilution II** are used in the test per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of neuronal transglutaminase (TG6).

The wells of the microtiter plate are coated with TG6. At the surface of the wells, the following immunological reactions take place:

First reaction: TG6 antibodies from the sample bind to the immobilised antigen forming the antigen-antibody complex. Afterwards, unbound sample components are washed from the microtiter plate.

Second reaction: A second antibody directed against human IgA and labelled with peroxidase (HRP) is added. This conjugate binds to the antigen-antibody complex. Excess conjugate is then washed from the microtiter plate.

Third reaction: The enzyme-labelled complex converts the colourless substrate in a coloured (blue) product. The extent of the colour development reflects the amount of TG6 antibody (IgA) present in the sample. Samples without TG6 antibodies remain colourless. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. Anti-hnTG IgA, present in the samples, is 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.	**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 standards/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** (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 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.
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

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.

Plasma and serum samples

Since the sample dilution is already considered in the calibration curve, the dilution factor is 1.

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 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*

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

LoB × *sample dilution factor to be used*

Analytical sensitivity × *sample dilution factor to be used*

Analytical sensitivity 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, instrument, day and kit lot).

Sample	Mean value [U/ml]	CV [%]
1	19.16	4.2
2	6.25	6.9

Reproducibility (Inter-Assay); n = 48

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

Sample	Mean value [U/ml]	CV [%]
1	18.84	7.1
2	5.79	7.7

Analytical sensitivity

Limit of blank, LoB 0.155 U/ml

12. PRECAUTIONS

- All reagents in the kit package are for *research* use only.

- 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 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.
- *IDK*® 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.

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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