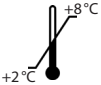
Manual

Immunglobulin G ELISA

For the determination of Immunoglobulin G in serum, plasma and urine

Valid from 2021-03-17

REF **KR6510A**



RUO

Immundiagnostik AG, Stubenwald-Allee 8a, 64625 Bensheim, Germany
Tel.: +49 6251 70190-0 Fax: + 49 6251 70190-363
e.mail: info@immundiagnostik.com www.immundiagnostik.com

Table of Contents

1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of Immunoglobulin G (IgG) in plasma, serum and urine. For research use only. Not for use in diagnostic procedures..

2. INTRODUCTION

Immunoglobulin G (IgG) is a subclass of antibodies which makes up the majority of antibodies found in blood. IgGs are glycoproteins with a molecular weight of about 150 kDa. They are part of the humoral immunity and are produced by B lymphocytes and plasma cells after contact with an antigen.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR6510A	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
KR6510A	CONJBUF	Conjugate dilution buffer, ready-to-use	1 x 22 ml
KR6510A	CONJ	Conjugate concentrate (rabbit-anti-IgG), peroxidase-labelled	1 x 200 µl
KR6510A	STD	Standards, lyophilised (see specification for concentrations)	2 x 5 vials
KR6510A	CTRL1	Control, lyophilised (see specification for range)	2 x 1 vial
KR6510A	CTRL2	Control, lyophilised (see specification for range)	2 x 1 vial
KR6510A	SAMPLE-BUF	Sample dilution buffer, ready-to-use	2 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

- 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** 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** as well as **concentrations and ranges** are given in the **specification data sheet**. **Standards and controls** (reconstituted STD and CTRL) **can be stored at 2–8 °C for 1 week or at -20 °C for 4 weeks. Avoid repeated thawing and freezing.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in **conjugate dilution buffer** (100 µl CONJ + 10 ml CONJBUF). The **CONJ** can be used until the expiry date stated on the

label when stored at **2–8 °C**. **Conjugate** (1:101 diluted CONJ) **is 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

Plasma or serum

Freshly collected EDTA plasma or serum can be stored for 2 weeks at 2–8 °C or for longer storage at -20 °C.

Urine

Adjust the urine to a pH of 6 to 8 with 1 N NaOH and store samples at 2-8°C until testing. For longer storage, samples should be frozen at –20 °C.

Sample dilution

Plasma or serum

EDTA plasma or serum samples must be diluted **1:200 000** in sample dilution buffer (SAMPLEBUF),before performing the assay. Dilution in three steps is recommended, e. g.:

- **10 µl** sample + **990 µl** SAMPLEBUF, mix well = **1:100** (**dilution I**)
- **10 µl** dilution I + **990 µl** SAMPLEBUF, mix well = **1:100** (**dilution II**)
- **50 µl** dilution II + **950 µl** SAMPLEBUF, mix well = **1:20** (**dilution III**). This results in a final dilution of 1:200 000.

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

Urine

Urine must be diluted **1:25** in sample dilution buffer (SAMPLEBUF) before performing the assay, e. g.:

e. g. **40 µl** sample + **960 µl** SAMPLEBUF, mix well.

100 µl of the dilution are used in the test.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of Immunoglobulin G (IgG) in plasma, serum and urine.

In a first incubation step, the Immunoglobulin G in the samples is bound to polyclonal rabbit antibodies (in excess) immobilised to the surface of the microtiter wells. After removal of all unbound substances, a peroxidase-labelled anti Immunoglobulin G antibody is added. The second washing step is followed by incubation with the substrate, tetramethylbenzidine (TMB). The reaction is terminated by an acidic stop solution converting the colour from blue to yellow. The intensity of the yellow colour is directly proportional to the concentration of Immunoglobulin G in the sample. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the results obtained from the calibrators. Immunoglobulin G, 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.

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** (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 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

The obtained results have to be multiplied by the **dilution factor 200 000** to get the actual concentrations.

Urine

The obtained results have to be multiplied by the **dilution factor 25** to get the actual concentrations.

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 with sample dilution buffer (SAMPLEBUF) 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

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

LoB 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 intra-assay variation was calculated from 20 determinations on each one of two urine or plasma samples measured by one person.

Sample	Mean value [mg/l]	CV [%]
Urine 1	4.18	3.94
Urine 2	1.94	4.13

Sample	Mean value [g/l]	CV [%]
Plasma 1	26.7	4.57
Plasma 2	11.3	2.67

Reproducibility (Inter-Assay); n = 12

The inter-assay variation was calculated from data on 2 plasma samples on different days. The samples have been measured by different technicians in 12 different assays.

Sample	Mean value [g/l]	CV [%]
1	14.00	6.48
2	17.94	3.85

Analytical Sensitivity

The following value has been estimated based on the concentrations of the standard without considering possibly used sample dilution factors.

The detection limit was set as B_0 + 1.645 SD. The Zero-standard was measured 84 times. The values were estimated in relation to the concentration of the calibration curve and resulted in

Limit of blank, LoB = 1.9 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 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 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.
- 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

Literature using Immunoglobulin G ELISA Kit [K6510A]

Cook S, Ladich E, Nakazawa G, Eshtehardi P, Neidhart M, Vogel R, Togni M, Wenaweser P, Billinger M, Seiler C, Gay S, Meier B, Pichler WJ, Jüni P, Virmani R, Windecker S (2009). Correlation of intravascular ultrasound findings with histopathological analysis of thrombus aspirates in patients with very late drug-eluting stent thrombosis. Circulation 4;120(5):391-9.

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