Manual

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Immunoglobulin G ELISA Kit

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

Valid from 07.02.2013

REF **K6510A**

Σ 96

+2 °C / +8 °C

IVD

1. INTENDED USE

The *Immundiagnostik* Assay is intended for the quantitative determination of **Immunoglobulin G (IgG)** in plasma, serum and urine. For *in vitro* diagnostic use only.

2. PRINCIPLE OF THE TEST

In a first incubation step, the Immunoglobulin G in the samples is bound to polyclonal rabbit antibodies (in excess) immobilized to the surface of the microtitre wells. After removal of all unbound substances, a Peroxidase-labeled 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 color from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of Immunoglobulin G in the sample. A dose response curve of the absorbance unit (optical density, OD) vs. concentration is generated using the results obtained from the calibrators. Immunoglobulin G in the patient samples is determined directly from this curve.

3. MATERIAL SUPPLIED

Cat. No	Content	Kit Components	Quantity
K6510AMTP	PLATE	One holder with precoated strips	12 x 8
K6510AWB	WASHBUF	ELISA wash concentrate, 10x	2 x 100 ml
K6510AK	CONJ	Conjugate, (rabbit-anti-IgG, Peroxidase-labeled)	1 x 200 µl
K6510AKV	CONJBUF	Conjugate dilution buffer, ready-to-use	1 x 22 ml
K6510AST	STD	Calibrators, lyophilized	2 x 5 vials
K6510AKO1	CTRL	Control, lyophilized	2 x 1 vial
K6510AKO2	CTRL	Control, lyophilized	2 x 1 vial
K6510APV	SAMPLEBUF	Sample dilution buffer ready-to-use	2 x 100 ml
K6510ATMB	SUB	TMB substrate (Tetramethylbenzidine) ready-to-use	1 x 15 ml
K6510AAC	STOP	ELISA stop solution, ready-to-use	1 x 15 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Precision pipettors calibrated and tips to deliver 10-1000 µl
- Covering foil for the microtiter plate
- Horizontal microtiter plate shaker with
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 nm
  (reference wave length 620 or 690 nm)

*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 < 0.055 µS/cm at 25°C (≥18.2 MΩ cm).

5. PREPARATION AND STORAGE 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 assay**. 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.
- The **WASHBUF** (wash buffer concentrate) should be diluted with ultra pure water **1:10** before use (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals can occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37°C in a water bath before dilution. The **WASHBUF** is stable at **2-8°C** until the expiry date stated on the label. **Diluted buffer solution** can be stored in a closed flask at **2-8°C for one month**.
- The lyophilized **STD** (standards) and **CTRL** (controls) are stable at **2-8°C** until the expiry date stated on the label. Standards and controls have to be reconstituted with **ultra pure water** (for standard and control concentrations as well as volume of ultra pure water, see product specification). Reconstituted calibrators and controls can be stored for one week at 4°C or for four weeks at -20°C. Repeated thawing and freezing should be avoided.

- The **CONJ** (conjugate) must be diluted **1: 101** in **CONJBUF** (Conjugate dilution buffer) (100 µl CONJ + 10 ml CONJBUF). The undiluted **CONJ** (conjugate) is stable at **2-8 °C** until the expiry date stated on the label. **Diluted conjugate is not stable and can not be stored.**
- All other test reagents are ready for use. The test reagents are stable up to the date of expiry (see label of test package) when stored at 2–8°C.

6. PRECAUTIONS

- For *in vitro* diagnostic 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.
- Reagents of the test package contain sodium azide as a bactericide. Contact with skin or mucous membranes must be avoided.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Reagents should not be used beyond the expiration date shown on the kit label.

7. SPECIMEN COLLECTION AND PREPARATION

Plasma or serum

Samples can be stored for two weeks at 2-8°C. For longer storage, samples should be frozen at –20°C.

Dilute all plasma and serum samples **1:200000** with SAMPLEBUF (sample dilution buffer)

Dilution in three steps is recommended.

For example:

1. **990 µl** SAMPLEBUF + **10 µl** sample **(1:100)**, mix well
2. **990 µl** SAMPLEBUF + **10 µl** of 1. dilution **(1:10000)**, mix well
3. **950 µl** SAMPLEBUF + **50 µl** of 2. dilution **(1:200000)**, mix well

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. Urine must be diluted **1:25** with SAMPLEBUF (sample dilution buffer) (960 µl SAMPLEBUF + 40 µl sample).

8. ASSAY PROCEDURE

Procedural notes

- Do not mix different lot numbers of any kit component. Furthermore, do not assemble cavities of different microplates for analyses, even if the microplates are of the same charge.
- Quality control guidelines 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 wrong use.
- The assay should always be performed according the enclosed manual.

Test procedure

Wash the pre-coated microtiter plate **5 x with 250 µl ELISA wash buffer**. Carry out the tests in duplicate. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess liquid.

1. Add **100 µl STD** (standard) **CTRL** (control) and **patient samples** (urine, plasma and serum diluted, see above).
2. Incubate for **1 hour** shaking on a horizontal mixer at room temperature (18-26°C).
3. Decant the content of the plate and wash the wells **5 x with 250 µl** ELISA wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess liquid.
4. Add **100 µl** of diluted **CONJ** (conjugate).
5. Incubate for **1 hour** shaking on a horizontal mixer at room temperature (18-26°C).

6. Decant the content of the plate and wash the wells **5 x with 250 µl** ELISA wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess liquid.
7. Add **100 µl** of **SUB** (TMB substrate solution).
8. Incubate for **10-20 minutes** at room temperature (18-26°C).
9. Add **100 µl** of **STOP** (stop solution) and mix shortly.
10. Determine absorption with an ELISA reader at **450 nm** against 620 nm as reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the measurement range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as reference.

9. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend to use the "4-Parameter-algorithm".

1. 4-parameter-algorithm

   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).

2. Point-to-point-calculation

   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

3. Spline-algorithm

   We recommend a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).

   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 control of the paired values should be done manually.

Serum/Plasma

The result must be multiplied by **200000** to calculate the serum value.

Urine

The result must be multiplied by **25** to obtain the IgG concentration in urine.

10. LIMITATIONS

Samples with IgG concentration greater than the highest standard value should be further diluted with sample dilution buffer and re-assayed.

11. QUALITY CONTROL

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Expected values

Normal range

Plasma or serum:	**8-18 g/L**
24-hours urine:	**0.5 – 3.2 mg/24 hours**
Spontaneous urine:	**< 10 mg IgG/g creatinine in urine**

For spontaneous urine samples, the IgG-values are normalized to the creatinine concentration in the urine.

It is recommended that each laboratory should establish its own normal range. Above mentioned values are only for orientation and may vary from other published data.

12. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay

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

Intra-Assay CV n= 20

Urine

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

Plasma

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

Inter-Assay

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.

Inter-Assay CV n= 12

Plasma

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

Detection limit

The calculated detection limit (LoB; Limit of Blank) was set as B_0 + 1.645*SD. Standard 1 (blank) was measured 84 times.

LoB = 1.9 ng/ml

The detection limit was estimated based on the concentration from the calibration curve without considering the sample dilution factor.

Recovery

Two Immunglobulin G-containing plasma samples were spiked with different Immunglobulin G-concentrations and measured.

Recovery n=5

Sample 1	**Spike [ng/ml]**	**Expected [ng/ml]**	**Measured [ng/ml]**
15.2	250.0	265.2	284.7
15.2	125.0	140.2	119.6
15.2	62.5	77.7	69.9
15.2	31.3	46.5	49.5
15.2	15.6	30.8	31.0
Sample 2	**Spike [ng/ml]**	**Expected [ng/ml]**	**Measured [ng/ml]**
22.0	250.0	272.0	258.9
22.0	125.0	147.0	124.5
22.0	62.5	84.5	80.3
22.0	31.3	53.3	48.4
22.0	15.6	37.6	39.0

Linearity

Two plasma samples were diluted with sample dilution buffer and measured with the assay.

Plasma

Sample	Dilution	Expected [g/L]	Measured [g/L]
A	1:100000	32.4	32.4
	1:200000	16.2	15.8
	1:400000	8.1	8.0
B	1:100000	17.1	17.1
	1:200000	8.6	9.6
	1:400000	4.3	5.5

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- All reagents in the test package are for *in-vitro* diagnostics only.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not to assemble wells of different microtiter plates for analysis, even if they are of the same batch.
- Quality control guidelines should be observed.
- 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 wrong use.

Used symbols:

	Temperature limitation		Catalogue Number
	In Vitro Diagnostic Medical Device		Contains sufficient for <n> tests
	Manufacturer		Use by
	Lot number		