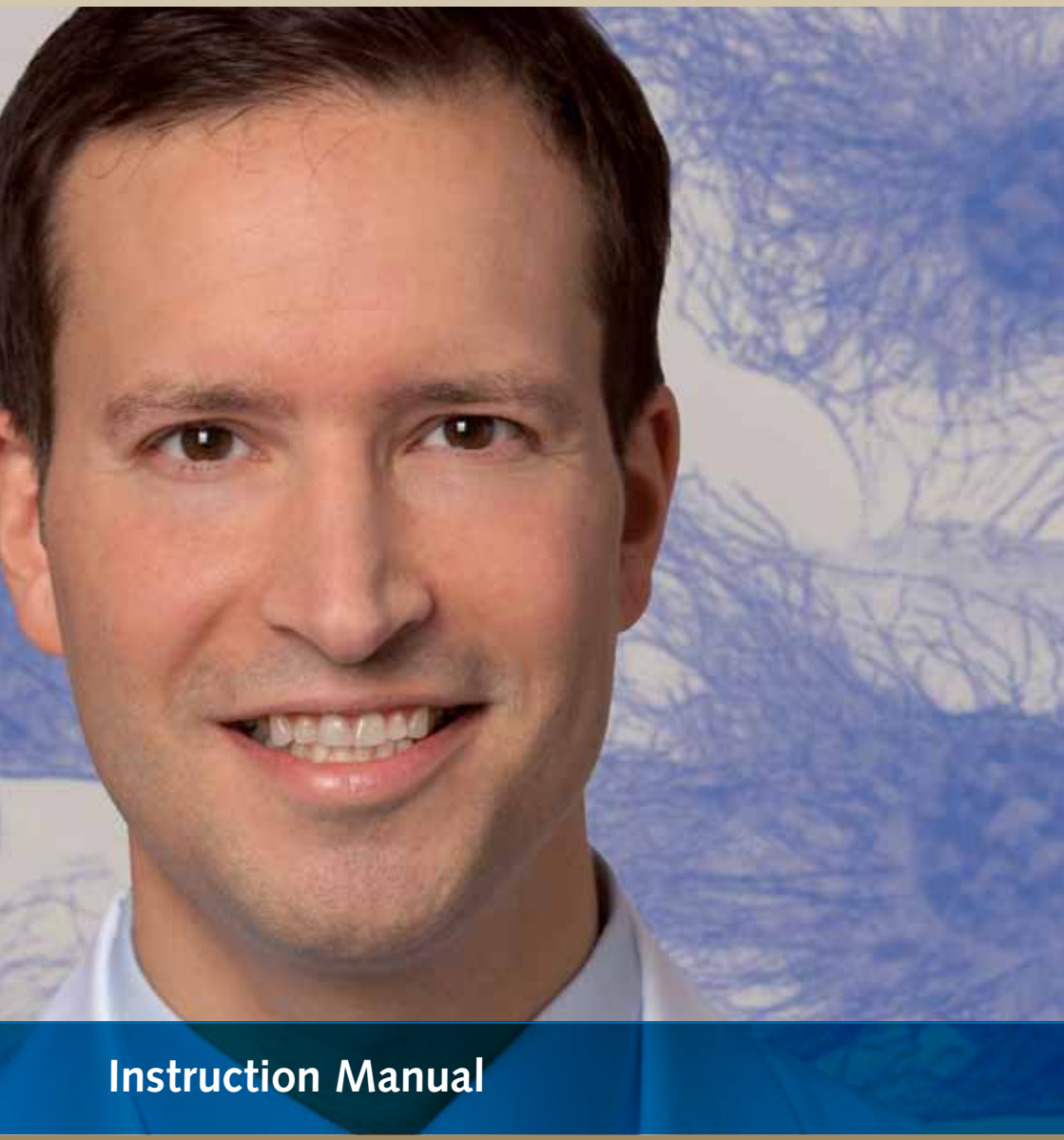
Human Immunglobulin G ELISA Kit



Instruction Manual

PromoKine

Contents

Intended Use

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

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 Peroxidaselabeled 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.

Material Supplied

Content	Kit Components	Quantity
PLATE	One holder with precoated strips	12 x 8
WASHBUF	ELISA wash concentrate 10x	1 x 100 ml
CONJ	Conjugate, (rabbit-anti-IgG, Peroxidase-labeled)	1 x 50 µl
CONJBUF	Conjugate dilution buffer, ready to use	1 x 22 ml
SAMPLEBUF	Sample dilution buffer, ready to use	2 x 100 ml
STD	Calibrators, lyophilized (0; 0.32; 0.8; 2; 5 mg/l)	5 x
CTRL	Control, lyophilized	1 x
NACL	0.9 % NaCl solution	1 x 30 ml
SUB	TMB substrate (Tetramethylbenzidine)	2 x 15 ml
STOP	ELISA stop solution, ready to use	1 x 15 ml

Material Required but not Supplied

- Ultra-pure water*
- Laboratory balance
- Precision pipettors calibrated and tips to deliver 10-1000 µl
- Covering foil for the microtiter plate
- Horizontal microtiter plate shaker with 37 °C incubator
- 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)

* Ultra-pure water (Water Type I; ISO3696) which is free of undissolved and colloidal ions and organic molecules (free of particles >0.2 µm) with an electric conductivity <0.055 µS/cm at 25°C (≥18.2 MΩ cm) is recommended.

Peparation 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 aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37°C in a water bath before dilution of the buffer solutions. The buffer concentrate 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 STD (Calibrators) and the CTRL (control) must be reconstituted with 250 µl ultra-pure water. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. Reconstituted calibrators and control can be stored at –20 °C until the expiry date given on the label. **Repeated thawing and freezing should be avoided**.

- The CONJ (conjugate) must be diluted 1: 1000 in CONJBUF (conjugate dilution buffer) (10 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.

Precautions

- For *in vitro* 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.
- 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.

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 **1:10,000** in two steps with **SAMPLEBUF** (sample dilution buffer).

For example:
1. **990 µl** SAMPLEBUF + **10 µl** sample **(1:100; Dilution I)**, mix well
2. **990 µl** SAMPLEBUF + **10 µl** of **dilution I (1:10,000; Dilution II)**, mix well
3. For analysis, pipette 10 µl of **Dilution II** per well.

Urine
Adjust the urine to a pH of 6 to 8 with 1 NaOH and store samples at 2-8°C until testing. For longer storage, samples should be frozen at –20°C. Samples with IgG-concentration higher than 5 mg/l must be diluted **1:10** with **SAMPLEBUF** (sample dilution buffer).

Assay Procedure

Procedural Notes
- Do not mix different lot numbers of any kit component.
- 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. PromoCell 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 precoated microtiter plate 5 x with 250 µl ELISA wash buffer (WASHBUF, 1:10 diluted). Carry out the tests in duplicate.

1. Pipette 200 µl of NACL (0.9% NaCl solution) into each well
2. Add 10 µl STD (standard), CTRL (control) or patient samples (urine, plasma and serum diluted, see above).
3. Incubate for 1 hour shaking on a horizontal mixer at room temperature.
4. Decant the content of the plate and wash the wells 5 x with 250 µl ELISA wash buffer.
5. Add 200 µl of diluted CONJ (conjugate).
6. Incubate for 1 hour shaking on a horizontal mixer at room temperature.
7. Decant the content of the plate and wash the wells with 5 x 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.
8. Add 200 µl of SUB (TMB substrate solution).
9. Incubate for 10-20 minutes at room temperature.
10. Add 50 µl STOP (stop solution) and mix shortly.
11. 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.

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 10,000 to calculate the serum value.

Urine (1:10 dilution)
The result must be multiplied by 10 to obtain the IgG concentration in urine.

Limitations

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

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

Plasma or serum:	8-18 g/l
Urine:	0.5 – 3.2 mg/24 hours

PromoCell recommends commercial control samples for internal quality control.

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.

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.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C and Australia antigen. 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.

- All reagents in the test package are for in-vitro research only.
- Reagents should not be used beyond the expiration date shown on the kit label.
- The test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- Do not interchange different lot numbers of any kit component within the same assay.
- 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. PromoCell can therefore not be held responsible for any damage resulting from wrong use.

Ordering Information

Product Name	Product Description	Size	Catalog Number
Immunglobulin G [IgG] ELISA Kit, human	Human Immunglobulin G [IgG] ELISA Kit	96 Tests	PK-EL-K6510

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

North America
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

Email: info@promokine.info