

# IMTEC-β<sub>2</sub>-GLYCOPROTEIN I-ANTIBODIES IgG

β<sub>2</sub>-GP1 IgG

ELISA for the Quantitative Determination of Anti-beta-2-Glycoprotein I Antibodies (IgG)

## Package Size

REF	ITC59150	96 Tests	Complete Test kit
IVD			

Please read the instructions carefully before testing.

### Procedural precautions:

Do not use the reagents beyond the date of expiry.

DIL DB03, WASH 20x WB06, SUB TMB ELISA and STOP STOP ELISA may be interchanged between lots and test kits that share the same reagent designation.

All other reagents are specific for the individual test kit lot and must not be interchanged with other lots and test kits.

Store reagents at 2...8°C.

## Intended Use

IMTEC-β<sub>2</sub>-Glycoprotein I-Antibodies IgG is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative determination of IgG class antibodies against beta-2-glycoprotein I in human serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of the antiphospholipid syndrome (APS).

Autoantibodies to beta-2-glycoprotein I (apolipoprotein H), are associated with arterial and venous thrombosis as well as recurrent spontaneous abortion.

Anti beta-2-glycoprotein I antibodies can be of the IgG, IgM and IgA-isotype. Especially those of the IgG isotype are associated with arterial and venous thrombosis.

## Indications:

- Suspicion of primary antiphospholipid syndrome (PAPS).
- SLE or other forms of connective tissue disease in conjunction with suspicion of secondary Antiphospholipid syndrome (SAPS)
- Risk assessment for thrombophilia and spontaneous abortion in case of APS.

## Principle

The test is based on the immobilisation of purified human beta-2-glycoprotein I to the solid phase of microtiter strips and subsequent binding of anti-beta-2-glycoprotein I antibodies from patient serum.

The bound antibodies are detected with a peroxidase-labelled secondary antibody that is directed against human IgG. After addition of substrate solution, a colour appears which intensity is proportional to the concentration and/or the avidity of the detected antibodies. Following the addition of stop solution, the colour switches from blue to yellow.

## Reagents and Contents

MTP	12	Microtiter Strips (in 1 strip holder) 8-well snap-off strips, ready for use coated with purified human beta-2-glycoprotein I
CAL	1 – 5 5 x 1.5 ml	Calibrators IgG (white cap), human serum, inked according to concentration, ready for use Level of anti-beta-2-glycoprotein I: 6.25 U/ml (1), 12.5 U/ml (2), 25 U/ml (3), 50 U/ml (4), 100 U/ml (5)
NC	1.5 ml	Negative Control Serum (green cap), human, ready for use
PC	1.5 ml	Positive Control Serum (red cap), human, ready for use Concentrations are stated on the labels.
WASH 20x WB06	50 ml	Washing Buffer (black cap) Concentrate (20x) for 1 l TRIS buffer pH 6.9 ± 0.2
DIL DB03	100 ml	Dilution Buffer (blue cap) ready for use Phosphate buffer pH 6.7 ± 0.2

++++ Change of  ++++ Please read **marked** text carefully! ++++

CON	15 ml	Conjugate Solution (white cap) anti-human-IgG HRP conjugate, ready for use
SUB TMB ELISA	15 ml	TMB solution (black cap) ready for use, colourless to bluish 3,3', 5,5'-tetramethylbenzidine Hydrogen peroxide pH 3.7 ± 0.2 1.2 mmol/l 3 mmol/l
STOP STOP ELISA	15 ml	Stop Solution (red cap) Sulphuric acid, ready for use 0.5 mol/l
	1	Adhesive Strip

## Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

## Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

## Reagent Preparation

**Allow the test kit and all its components to reach room temperature before use!** Used bottles should be closed carefully and stored at 2...8°C. Store SUB protected from light.

Do not use polystyrene vessels for handling of CON.

To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

## Washing Buffer Solution WASH

Any crystallised salt inside the bottle must be resolved before use. Dilute 1 part WASH 20x with 19 parts distilled water. WASH is stable for 6 weeks stored at 2...8°C.

## Specimen

Patient sera or plasma

Use samples freshly collected or freeze samples at –20°C. **Freeze and thaw once only.** Do not use serum samples inactivated by heat treatment at 56°C.

Allow the samples to reach room temperature (30 min).

Dilute samples 1:101 with DIL (add 10 µl sample to 1 ml DIL).

## Procedure

- Pipette 100 µl diluted sample, CAL, PC and NC into MTP, for blank use DIL instead of sample dilution, seal MTP with adhesive strip.
- Incubate for 1 hour at RT.
- Discard the solution from MTP. Wash MTP 3 times using 300 µl WASH per well.
- Discard WASH and knock out residues on an absorbent paper or cloth.
- Pipette 100 µl CON and seal MTP with adhesive strip.
- Incubate for 30 min. at RT.
- Discard the solution from MTP. Wash MTP 3 times using 300 µl WASH per well.
- Discard WASH and knock out residues on an absorbent paper or cloth.
- Pipette 100 µl SUB and incubate for 10 min.. At room temperatures above 25°C the substrate incubation could be shortened, but should never fall short of 5 min..
- Add 100 µl STOP per well.
- Read absorbance values at 450 nm within the next 10 min. after stopping. Bi-chromatic measurement with a reference wavelength at 620 – 690 nm is recommended..

## Automation

The IMTEC-β<sub>2</sub>-Glycoprotein I-Antibodies IgG ELISA may be processed with suitable automated ELISA analyzers. Applications have to be validated prior to diagnostic use.

## Validation of the Test

The test results are valid provided the following criteria are met for the obtained results:

- **PC** is within the indicated range (see label).
- **NC** is lower than the cut-off-value of the test.
- **CAL** **5** does not fall below an absorbance value of 0.6.
- The absorbances of **CAL** **1**-**5** keep raising.

In order to improve accuracy of the test results we recommend to run **CAL** **1**-**5**, **PC**, **NC** and patient samples in duplicate.

## Interpretation of Results

Plot measured absorbances against U/ml of **CAL** **1**-**5** in semi-log. By interpolating the plotted measuring points, a calibration curve is obtained, from which the concentrations of anti-beta-2-glycoprotein I antibodies in the patient samples can be determined.

Results above 7 U/ml (cut-off value) for anti-beta-2-glycoprotein I IgG-antibodies are considered positive.

## Limitations

A positive result must be used in association with clinical evaluation and diagnostic procedures. The values obtained from this assay are intended to be an aid for diagnosis only.

Elevated anti-beta-2-glycoprotein I antibodies may occur in individuals with no evidence of clinical disease.

If the patient sample contains elevated levels of immune complexes or other immunoglobulin aggregates, false positive results by non-specific binding cannot be ruled out.

## Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via:

[www.human.de/data/gb/vr/el-59150.pdf](http://www.human.de/data/gb/vr/el-59150.pdf) or

[www.human-de.com/data/gb/vr/el-59150.pdf](http://www.human-de.com/data/gb/vr/el-59150.pdf)

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

## Safety Notes

### **STOP** Warning

#### • Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

### **SUB** Danger

#### • Hazard statements

H360D May damage the unborn child.

#### • Precautionary statements

**CAL** **NC** **PC** **WASH** **20x** **DIL** **CON** **SUB** **STOP**

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

## References

1. Mackworth-Young C. G. *et al.*, Clin. Exp. Immunol. **136**, 393-401 (2004)
2. Cervera R. *et al.*, Arthr. Rheum. **46**, 1019-1027 (2002)
3. Miyakis S. *et al.*, J. Thromb. Haemost. **4**, 295-306 (2006)
4. Luzzana C. *et al.*, J. Nephrol. **15**, 342-348 (2002)
5. Empson M. *et al.*, Cochrane Database Syst. Rev. **18**, CD002859 (2005)
6. Lassere M., Empson M., Thromb. Res. **114**, 419-426 (2004)
7. Conrad K. *et al.*, Autoantikörper bei systemischen Autoimmunerkrankungen - ein diagnostischer Leitfaden, Pabst Sci. Publ., Lengerich, 48-50 (2006)

EL-59150

INF ITC59150 GB

08-2018-13M



# Human