BlueWell

β_2-Glycoprotein I IgG ELISA

Order Code: GPG02-96

96 test quantitative Enzyme Immunoassay

1. INTENDED USE

The BlueWell β_2–Glycoprotein I IgG ELISA kit allows the quantitative detection of IgG antibodies to β_2–Glycoprotein I in human serum.

2. PRINCIPLE OF THE TEST

The BlueWell β_2–Glycoprotein I IgG kit is a solid phase enzyme immunoassay using 96 coated breakaway microwells and a peroxidase-TMB detection system. The microwells are coated with highly specific antigen.
In the test procedure, serum samples are diluted 1/51 and incubated in the microwells. Human antibodies, if present, bind to the specific antigen. Unbound or excess antibodies are removed by washing and HRP-conjugated rabbit antibodies against human IgG are added to the microwells. The enzyme conjugate binds to the antigen-antibody complexes. After a second washing step to remove excess conjugate, the TMB/substrate solution is added. The enzyme activity, if present, generates a colorimetric (blue) reaction. Diluted acid is added to stop the reaction. Consequently the colour turns from blue to yellow and may be measured at 450 nm using a conventional microplate reader. The absorbance (Optical Density) is directly proportional to the concentration of IgG antibodies bound to the antigen on the microwells surface

3. KIT CONTENTS

3.1 Material provided in the kit

<u>To be reconstituted</u>: **20x Wash Buffer**	**1 vial, 50 ml - 20 x concentrated (blue)** *Containing: Tris, Tween, Methylisothiazolone (preservative)*
<u>Ready to use</u>: **Sample Diluent**	**1 vial, 50 ml (yellow)** *Containing: Tris, Tween, BSA, Methylisothiazolone (preservative)*
Substrat	**1 vial, 20 ml (colourless)** *Containing: stabilised TMB/H_2O_2, Methylisothiazolone (preservative)*
Negative control	**1 vial, 1 ml (green)** *Containing: human serum (diluted), Methylisothiazolone (preservative)*
Calibrated standards	**6 vials, 1 ml each 0, 25, 50, 100, 200, 400 U/ml.** **(colour increasing with concentration)** *Containing: human serum (diluted), Methylisothiazolone (preservative)*
Positive control	**1 vial, 1 ml (blue)** *Containing: human serum (diluted), Methylisothiazolone (preservative)*
Conjugate	**1 vial, 20 ml (red)** *Containing:Rabbit anti-human IgG/peroxidase, Methylisothiazolone (preservative)*
Stop solution	**1 vial, 20 ml (colourless)** *Containing: sulfuric acid 2.5 %*
Microwell strips	**12 x 8 well strips with breakaway microwells** *Coated with purified β_2–Glycoprotein I (purified, human)*
Frame for strips	**1**

3.2 Material required but not provided

- Microtiter plate reader (450 nm reading filter + optional 650 nm reference filter)).
- Glass ware, test tubes for the dilutions.
- Distilled water.
- Precision pipettes (10, 100, 200, 500, 1000 µl) or multipipette.
- Microplate washing device (multichannel pipette or automated system)
- Absorbent paper.

4. STORAGE AND SHELF LIFE

- Store all reagents and microwells at 2-8°C
- Once prepared (refer to 7.2), the washing solution is stable for 1 month at 4°C.
- Reagents and microwells should be used until the expiry date indicated on each component only.

5. PRECAUTIONS OF USE

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE AND PROFESSIONAL USE ONLY.

Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following recommendations and precautions for maximum safety when handling:

- The kit contains potentially hazardous components. Reagents may be irritating to eyes and skin thus avoid contact with eyes and skin. Do not smoke, eat or drink when manipulating the kit.
- All human source material used for some reagents of this kit (controls, standards) has been tested and found negative for HbsAg, for Hepatitis C and for HIV 1 and 2 antibodies by approved methods. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases.

5.2. Other precautions

- Do not mix or substitute reagents or microwells from different lot numbers. This may lead to variations in the results.
- Allow all components to reach room temperature (18-24C) before use and follow the recommended incubation scheme for an optimimum performance of the test
- Always pipette reagents with clean tips in order to avoid contamination with exogenous substances.
- Protect the chromogen / substrate reagent from light to avoid increase in blank values.

6. SAMPLE COLLECTION, HANDLING AND STORAGE

- Use preferentially freshly collected serum samples.
- Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be clarified by low speed centrifugation.
- Blood samples should be collected in dry tubes. After separation, the serum samples should be used immediately, respectively stored at 2-8°C for two or three days, or frozen at -20°C for longer periods.

7. ASSAY PROCEDURE

7.1 Samples

- Dilute serum samples **1:51** with sample diluent (ready-to-use)
  → **e.g. 500 µl** diluent + **10 µ**l serum. **Mix.**

7.2 Wash buffer

- Dilute the concentrated Wash buffer **1:20** with distilled water

◇ ***Manual washing:*** Prepare **10 ml** final volume per **8 wells** or **120ml** for **96 wells**
→ **e.g. 9.5 ml** water + **0.5 ml** buffer. Mix.

◇ ***Automated washing:*** consider excess volumes required for setting up the instrument and dead volume of robot pipette.

7.3 Microwells

- Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store them in the provided plastic bag, sealed tightly

7.4 Pipetting Scheme

- Make sure all reagents are at room temperature before use (18-24°C)

- **Pipette 100 µl** of each patient's **diluted serum** into the designated microwells.
- **Pipette 100 µl standards and controls** into the designated wells.
- **Incubate** for **30 minutes** at room temperature (18-24°C).
- **Wash 3 X** with **200 µl washing buffer (diluted 1:20)**.
- **Pipette 100 µl conjugate** into each well.
- **Incubate** for **30 minutes** at room temperature (18-24°C).
- **Wash 3 X** with **200 µl washing buffer (diluted 1:20)**.
- **Pipette 100 µl substrate** into each well.
- **Incubate** for **10 minutes** at room temperature (18-24°C).
- **Pipette 100 µl stop solution** into each well, using the same order as pipetting the substrate.
- **Read absorbance** at **450 nm** (optionally 450/650 nm) within 30 minutes.

NOTE: We recommend to pipette a blank in duplex with each run. (sample diluent only, instead of a patient's sample)

Manual washing procedure
Discard liquid from wells by inverting the plate. Knock the microwell frame with wells down-sided vigorously on clean absorbent paper. Pipette 200 µl of diluted wash buffer into each well, wait for 20 seconds, repeat discard and knocking. Repeat the whole procedure twice again.

8. CALCULATION AND INTERPRETATION OF THE RESULTS

8.1 Quantitative interpretation

Establish the standard curve by plotting the optical density of each standard with respect to the corresponding units values. For best results we recommend lin/lin algorithm. From the O.D. of each sample, read the corresponding antibody concentrations expressed in U/ml.

Normal Range: IgG ≤ 25 U/ml

INTERPRETATION	**Negative result**	**Positive result**
	≤ 25 U/ml	**> 25 U/ml**

NOTE: Borderline samples should be tested again for confirmation.

8.2 Semi-quantitative interpretation

A semi-quantitative interpretation of the results is available by using the **25 U/ml** standard as a cut off control. Results are expressed in **B**inding **I**ndex, the ratio between the sample and the cut off's O.D.:

B.I. = Sample O.D / Cut-off O.D

A sample is **negative** when **B.I. ≤ 1.0**
A sample is **positive** when **B.I. > 1.0**
NOTE: Borderline samples should be tested again for confirmation.

8.3 Validation of results

A test run is considered valid if the following Quality Assurance specifications are met.
If not, refer to § 11, check the whole procedure and repeat the test. If the problem persists call manufacturer or distributor for assistance.

	Quality Assurance specifications	
	O.D.	**U./ml**
Blank (sample diluent)	< 0.100	-
Negative control	-	≤ 20
25 U/ml Standard	< 50 % of Standard 400 U/ml	-
Positive control	> 0.800	200 – 400

9. PERFORMANCES

9.1 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies individual samples may not follow this rule in every case. Detailed and updated data are available upon request.

9.2 Reproducibility

Three control sera (High, medium, low) were assayed for intraassay and interassay imprecision in a statistically relevant repetition. The variation coefficients are <10% intra- and <20% inter-lot. Detailed and updated data are available upon request.

9.3 Clinical Sensitivity and Specificity

Sensitivity is estimated to be 97.3 %
Specificity is estimated to be 96.0 %
Clinically defined populations (confirmed positive with disease specific reference methodologies) have been used for checking the sensitivity. Specificity was checked with control groups that embrace a normal healthy population as well as clinically defined control groups. Detailed data are available upon request.

9.4. Expected Values

The expected value for a normal patient is a negative result. The number of positives, and the degree of positivity is dependent upon parameters such as population type being tested, treatment, etc. Each laboratory should consequently establish its own expected values based upon the specimens typically being tested.

10. TEST LIMITATIONS

1. A diagnosis should not be made solely on the basis of the test results.
2. Test results should always be interpreted in conjunction with the complete clinical evaluation and the results of other diagnostic procedures, only.
3. D-tek s.a. and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
4. In any case, GLP should be applied with all general and individual regulations to the use of this kit.

11. BASIC TROUBLE SHOOTING

Optical density too low	**Optical density too high**
Please, check the following possibilities: • Inappropriate reader filter (use 450 nm or 450/650nm) • Correct dilution of washing buffer (under-diluted) • Correct dilution of samples (over-diluted) • Inactivation of conjugate (by exogenous substances e.g.). Use clean tips only.	Please, check the following possibilities: • Insufficient washing (See manual washing procedure in & 7.4) • Excess incubation time or temperature • Correct dilution of samples (under-diluted) • Contamination of substrate reagent (by conjugate e.g. → color obviously blue already in the bottle). Use clean tips only. • Contamination of samples (by micro-organisms e.g.). Use preferentially fresh samples.

12. BIBLIOGRAPHY

Up to date literature is available upon request. Please inquire at info@d-tek.be

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