Human DGP / Deamidated gliadin proteins IgA antibody ELISA Kit

Enzyme Immunoassay for the quantification of IgA class antibodies against DGP / Deamidated gliadin proteins in human serum or plasma

Catalog number: ARG81022

For research use only. Not for use in diagnostic procedures.

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

PRINCIPLE OF THE ASSAY

This assay employs the qualitative enzyme immunoassay technique. DGP / Deamidated gliadin antigen have been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, an HRP-conjugated anti-human-IgA is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of IgG Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Calibrator A-F (0, 6.3, 12.5, 25, 50, 100 U/mL)	6 X 1.5 ml (Ready-to-use)	4°C
Positive Control	1.5 ml (Ready-to-use)	4°C
Negative Control	1.5 ml (Ready-to-use)	4°C
HRP-conjugated Anti-human-IgA	15 ml (Ready-to-use)	4°C
50X Wash buffer	20 ml	4°C
5X Sample Diluent	20 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)

STOP solution	15 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm/620 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Vortex tube mixer
- 37°C incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times and do not use after the expiry date.
- After the first opening the kit should be used within 3 months, the diluted wash buffer can be kept for 4 weeks at 4°C.
- If crystals are observed in the 50X Wash buffer, warm up to 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- The TMB solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.
- It is very important to bring all reagents and samples to room temperature (20-25 °C) and mix them before starting the test run.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately. The samples can be stored at 2-8 °C up to a week or aliquot and store samples at-20°C or -80°C for longer storage. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, citrate or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately. The samples can be stored at 2-8 °C up to a week or aliquot and store samples at-20 °C or -80°C for longer storage. Avoid repeated freeze-thaw cycles.

Note:

1. If samples are stored frozen, mix thawed samples well before testing.
2. Heat inactivation of samples is not recommended.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. Mix well. (e.g. 20 ml of 50X wash buffer + 998 ml of distilled water) The diluted 1X wash buffer is stable for 4 weeks at 2-8°C.
- 1X Sample Diluent: Dilute 5X Sample Diluent into distilled water to yield

1X Sample Diluent. Mix well. (e.g. 20 ml of 5 X Sample Diluent + 80 ml of distilled water) The diluted 1X Sample Diluent is stable for 4 weeks at 2-8°C.

- **Patient sample**: Dilute patient sample 1:100 with 1X sample buffer before assay, mix well. (e.g. 10 µl of sample + 990 µl of 1X sample buffer)
  Note: the controls and calibrators are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25 °C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal it and store at 2-8°C.
2. Add 100 µl of **1:100 diluted** samples, Calibrators and controls (undiluted, ready-to-use) into wells. Leave one well empty for the substrate blank.
3. Cover the plate and incubate for 30 minutes at room temperature.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Keep the buffer in the well for at least 10 sec before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl HRP-Anti-human-IgA solution (ready-to-use) into each well (expect the substrate blank wells). Incubate for 15 minutes at RT.

6. Wash as according to step 4.
7. Add 100 µl of TMB Reagent to each well (including the well for substrate blank). Cover the plate and incubate for 15 minutes at RT in dark.
8. Add 100 µl of Stop Solution to each well in the same order and at the same rate as for the TMB Substrate Solution (including substrate blank wells).
9. Incubate for 5 minutes at RT in dark.
10. Read the OD with a microplate reader at 450 nm immediately. (600-690 nm as optional reference wave length) and use the substrate controls as blank. The color is stable for at least 30 minutes.

CALCULATION OF RESULTS

1. For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve.
2. The concentration of patient samples may then be estimated from the cali-bration curve by interpolation.
3. Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and con-centration is the data reduction method of choice.

QUALITY ASSURANCE

Sensitivity

Functional sensitivity was determined to be: 1 U/ml.

Measuring Range

0-100 U/mL

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 10 U/ml

Interpretation of results

Negative: < 10 U/ml

Positive: ≥ 10 U/ml

Clinical Specificity

100%

Clinical Sensitivity

70%

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.0-6.0% and inter-assay precision was 1.7-8.7%.

Linearity

81 – 100 %

Interferences

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglyc-erides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfer-ing effects been observed

with the use of anticoagulants (Citrate, EDTA, Heparin). However for practi-cal reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.