Human dsDNA antibody IgG ELISA Kit

Enzyme Immunoassay for the quantification of IgG antibodies to dsDNA in serum or plasma.

Catalog number: ARG80382

Package: 96 wells

TABLE OF CONTENTS

SECTION **Page**

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. Human recombinant ds-DNA has 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, a HRP-conjugated anti human antibody 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 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.
Standard A-F (0, 12.5, 25, 50, 100, 200 IU/ml)	6 x 1.5 ml (Ready-to-use)	4°C.
Control 1 (60-90 IU/ml)	1.5 ml (Ready-to-use)	4°C.
Control 2 (<10 IU/ml)	1.5 ml (Ready-to-use)	4°C.
HRP-Antibody conjugate	15 ml (Ready-to-use)	4°C

50X Wash buffer	20 ml	4°C
5X Sample Buffer	20 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 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.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 50X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- 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 or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer.
- 1X Sample Buffer: Dilute 5X Sample buffer with distilled water before use.
- 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 standard are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) 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, and reseal it.
2. Add **100 µl** of **controls**, **diluted samples** and **Standard into** wells.
3. Incubate for 30 minutes at RT.
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. 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 1X Antibody solution** into each well. Incubate for 15 minutes at RT.
6. Wash as according to step 4.
7. Add **100 µl** of **TMB Reagent** to each well. Incubate for 15 minutes at RT.
8. Add **100 µl** of **Stop Solution** to each well. Incubate for 5 minutes at RT. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may be estimated from the calibration curve by interpolation.

Using data reduction software a 4-parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

INTERPRETATION OF RESULTS

In a normal range study with samples from health blood donors the following ranges have been established with this ELISA assay: Cut-off 20 IU/ml

Negative: OD of sample < 20 IU/ml

Positive: OD of sample ≥ 20 IU/ml

QUALITY ASSURANCE

Limit of detection

Functional sensitivity was determined to be 1 IU/ml.

Interference

No interference has been found with the following factors:

Hemolytic or lipemic sera, bilirubin, anticoagulants (Citrate, EDTA, Heparin).

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.7% and inter-assay precision was 8.3%.