Protocol - Human anti-DS-DNA-IgG antibodies ELISA

Catalog #: 60812

For the quantitative measurement of Human anti-DS-DNA-IgG antibodies in serum, plasma, supernatants.

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

Introduction

This ELISA is a solid-phase indirect enzyme-linked immunosorbent assay (ELISA) for the quantitative measurement of Human anti-DS-DNA-IgG antibodies in biological samples. This assay is highly specific and sensitive, providing a reliable method for quantitative analysis.

Principle of the Assay

This assay employs an indirect ELISA for the quantitative measurement of Human anti-DS-DNA-IgG antibodies. Samples and Standards are added to antigen-coated microplate wells, allowing target-specific binding molecules to bind to the immobilized antigen. After washing, an HRP-labeled secondary antibody is added to detect the bound molecules. Following a final wash, Substrate Solution (TMB) is added, producing a colorimetric reaction proportional to the amount of bound analyte. The reaction is stopped with Stop Solution, and absorbance is measured to calculate concentrations from the Standard Curve.

Materials Provided & Storage Conditions

Component	Description
ELISA Microplate	96-well (12 strips × 8) coated microplate
Lyophilized Standard	2 vials
HRP-Detector Reagent (100X)	120 µL
Sample Dilution Buffer	20 mL
HRP-Detector Dilution Buffer	10 mL
Wash Buffer Concentrate (25X)	30 mL
Substrate Solution (TMB)	10 mL
Stop Solution	10 mL
Plate Sealers	5 adhesive sealing films

* Store the unopened kit at 2–8°C. Do not use past the expiration date. Do not combine materials from different lots.

Other Supplies Required

- Microplate reader capable of absorbance measurements at 450 nm, with optional correction at 540 nm or 570 nm
- Adjustable single-channel and multichannel pipettes with compatible pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 37°C incubator; do not use a humidified cell culture incubator

Precautions & Technical Hints

- Add all reagents in the same order and at consistent time intervals to minimize variation between wells.
- To avoid cross-contamination, use fresh pipette tips for each Standard, sample, and reagent.
- Use a multichannel pipette for rapid dispensing. Use fresh multichannel reservoirs for dispensing reagents to prevent cross-contamination, especially for Substrate Solution (TMB).
- Substrate Solution (TMB) should be colorless before addition. Protect from light.
- Stop Solution is acidic. Treat all biological materials as potentially biohazardous. Wear protective gloves, clothing, and eye protection.

Sample Collection & Storage

The following are general guidelines. Sample stability has not been fully characterized.

- **Cell Culture Supernatants:** Remove particulates by centrifugation at 1000 × g for 15 minutes. Assay immediately or aliquot and store samples at ≤ -20°C.
- **Serum:** Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C.
- **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C.
- **General Note:** Avoid repeated freeze-thaw cycles for all samples.

Reagent Preparation

Bring all reagents to room temperature before use. 1X Wash Buffer may be prepared in full at any time. Prepare only the required volume of 1X HRP-Detector Reagent, no more than 30 minutes before use.

- **1X Wash Buffer:** If crystals are present, warm to room temperature and mix gently until dissolved. Dilute 30 mL Wash Buffer Concentrate (25X) with 720 mL deionized water to prepare 750 mL 1X Wash Buffer.
- **1X HRP-Detector Reagent:** Dilute HRP-Detector Reagent (100X) 1:99 in HRP-Detector Dilution Buffer. Prepare enough for 100 µL per assay well.

Sample and Standards Preparation

Prepare diluted samples and the Standard dilution series. Use all diluted Standards and samples within 2 hours. A second vial of Lyophilized Standard is provided for later experiments.

- **Samples:** Dilute samples in Sample Dilution Buffer. Matrix effects may influence assay performance; testing multiple sample dilutions is recommended. Serial dilutions may be required for high dilution factors.
- **Standard #1:** Add 1 mL Sample Dilution Buffer to the Lyophilized Standard vial. Mix gently by inversion, let stand for 2 minutes, and label as Standard #1.
- **Standards #2–7:** Label six tubes Standard #2–Standard #7, each with 0.3 mL Sample Dilution Buffer. Transfer 0.3 mL from Standard #1 to Standard #2 and mix gently by inversion. Repeat through Standard #7.
- **Standard #8:** Prepare a blank Standard tube labeled Standard #8 containing 0.3 mL Sample Dilution Buffer only.

Assay Procedure

1. Bring all reagents and samples to room temperature. Prepare Standards, 1X Wash Buffer, 1X HRP-Detector Reagent, and appropriately diluted samples. Place the required number of microplate strips into the plate frame. Duplicate Standard and sample measurements are recommended. Return unused strips to the foil pouch containing the desiccant pack, and reseal.
2. Add 100 µL of each Standard to the designated wells. Add 100 µL of each sample to the designated wells. Record sample positions. Apply a plate sealer and incubate at 37°C for 90 minutes.
3. Aspirate the contents of each well and wash three times with 350 µL of 1X Wash Buffer per wash. Ensure complete removal of liquid at each step. After the last wash, remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
4. Add 100 µL of 1X HRP-Detector Reagent to all wells. Apply a plate sealer and incubate at 37°C for 60 minutes.
5. Aspirate the contents of each well and wash five times with 350 µL of 1X Wash Buffer per wash. Wash and blot the plate as described above.
6. Add 90 µL of Substrate Solution (TMB) to all wells. Incubate at 37°C, protected from direct light, for 10–20 minutes. Monitor color development. The reaction may be stopped when an appreciable color gradient is observed across the Standard dilution series.
7. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. Gently tap the plate to mix the contents of each well.
8. Measure the Optical Density (OD) of each well within 30 minutes using a microplate reader set to 450 nm. If wavelength correction is available, set correction to 540 nm or 570 nm.

Calculation of Results

- Average duplicate readings for each Standard and sample, then subtract the mean OD of the blank Standard.
- Create a Standard Curve by plotting the mean absorbance for each Standard on the y-axis against the concentration on the x-axis. A four-parameter logistic (4PL) curve fit is recommended.
- A Standard Curve may be generated manually, with spreadsheet software, with microplate reader software, or using our provided tool: https://benchmarkantibodies.com/graphing/
- If samples have been diluted, the concentration read from the Standard Curve must be multiplied by the dilution factor.

Performance Characteristics

Standard Curve

Standard curve Human DS-DNA-IgG ELISA Kit 60812 [ng/mL]

Stability

37°C / 1 Month	2-8°C / 6 Months	2-8°C / 12 Months
80	95-100	85-98