High Avidity ds-DNA IgG antibodies ELISA

Quantitative assay for high avidity antibodies to double stranded DNA

Product code GD128 96 Tests

CE

1. Intended use

The high avidity ds-DNA IgG antibodies kit is a rapid quantitative ELISA method for the detection of high avidity IgG antibodies to double-stranded (ds) DNA. It is intended as an aid to diagnosis of systemic lupus erythematosus (SLE) and as a tool to monitor the clinical course of the disease in individual patients. The components of the kit are for *in vitro* diagnostic use only.

2. Explanation of the Test

Circulating antibodies to dsDNA are strongly associated with systemic rheumatic diseases, including SLE. Importantly, the avidity of these antibodies is variable and only high avidity anti-dsDNA antibodies are diagnostic of SLE. The Farr radioimmunoassay is regarded as the gold standard for the detection of high avidity anti-dsDNA antibodies. However, this assay uses the radioisotope I^{125}, is time-consuming and also detects IgM autoantibodies which occur in non-SLE diseases. Genesis high avidity DNA antibody kit is a modification of Genesis broad spectrum dsDNA IgG ELISA which detects both high and lower affinity antibodies. The modified assay has a diagnostic specificity for SLE of 100%.

3. Principle of the test

Diluted serum samples are incubated with dsDNA immobilised on microtitre wells. After washing away unbound serum components, rabbit anti-human IgG conjugated to horseradish peroxidase is added to the wells, and this binds to surface-bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of stop solution terminates the reaction and provides the appropriate pH for colour development. The optical densities of the standards, positive control and samples are measured using a microplate reader at 450nm.

4. Materials included in the kit

- **Microplate:** 96 wells in 12 X 8 break-apart strips, pre-coated with human dsDNA, with holder in a foil bag with desiccant
- **Reagent 1**: **Sample Diluent** 150mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 10ml, (blue), concentrate (x15)
- **Reagent 2: Wash Buffer** 50mM Tris-buffered saline with detergent, pH 7.2, 100 ml, **concentrate (x5)**
- **Reagent 3: Conjugate** rabbit anti-human IgG conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 12 ml, (red), ready to use
- **Reagent 4: TMB Substrate** aqueous solution of TMB and hydrogen peroxide, 12 ml, ready to use
- **Reagent 5: Stop Solution** 0.25M sulphuric acid, 12 ml, ready to use
- **Standards:** 0, 25, 50, 100, 200 & 400 IU/ml[1], 1ml of 10mM Tris-buffered saline containing human serum IgG antibodies to dsDNA, ready to use
- **Positive Control:** 1ml of 10mM Tris-buffered saline containing human serum antibodies to dsDNA, ready to use
- **Instructions for use**

[1] Calibrated against IRP Wo/80

5. Other equipment required

1. Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 10µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable automated system may be used.
2. Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

6. Precautions

6.1 Safety Precautions

1. All reagents in this kit are for *in vitro* diagnostic use only.
2. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
3. All human source material used in the preparation of standards and the positive control for this product have been tested and found negative for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
4. Reagents of this kit contain antimicrobial agents and the Substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
5. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
6. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local legislation.

6.2 Technical Precautions

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. If crystals are present in the wash buffer, allow to dissolve before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 – 8°C after use.
3. The sample diluent X15 concentrate contains 0.09% sodium azide as preservative. Prepare sufficient working strength diluent for the assay run. However, if the working strength diluent is to be stored for more than 1 week, add sodium azide (0.9g/L). Store unused sample diluent concentrate and dilute sample diluent at 2 – 8°C.
4. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette
5. Include the Positive Control in every test run to monitor for reagent stability and correct assay performance.
6. Strictly observe the indicated incubation times and temperature.
7. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate reagent.
8. When pipetting Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
9. Do not allow microwells to dry between incubation steps.
10. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.
11. Avoid direct sunlight and exposure to heat sources during all incubation steps.
12. Replace colour-coded caps on their correct vials to avoid cross-contamination
13. It is important to dispense all samples and the positive control into the wells without delay. Therefore ensure that all samples are ready to dispense.

7. Shelf life and storage conditions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for 3 months (or until its expiry date if less than 3 months). Do not use kits beyond their expiry date. Do not freeze any kit component. The diluted Wash Buffer and diluent has a shelf life of 3 months (see Technical Precautions) if stored in a closed bottle at 2 – 8°C.

8. Specimen collection and storage

Serum or plasma samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

9. Preparation of reagents

1. Dilute the Sample Diluent **(Reagent 1)** 1:14 in distilled water to make sufficient buffer for the assay run e.g. add 10ml sample diluent concentrate to 140 ml water.

2. Dilute the Wash Buffer (**Reagent 2)** 1: 4 in distilled water. **PLEASE NOTE THE WASH BUFFER IS A X5 CONCENTRATE.** So, for example, add 100ml wash buffer concentrate to 400ml distilled/deionised water and mix thoroughly.

10. Assay Procedure

1. Dilute patient samples 1:100 in diluted Sample Diluent (e.g. 10µl serum plus 1ml diluent).

2. Assemble the number of strips required for the assay.

3. For quantitative assays, dispense 100 µl of each Standard, the Positive Control and the diluted patient samples into appropriate wells. For qualitative assays, dispense only the 50 IU/ml Standard together with the positive control and samples.

4. Incubate for **30** minutes at room temperature.

5. After 30 minutes, decant or aspirate the well contents and wash the wells 3 times using automated washing or the manual wash procedure (see below). Careful washing is the key to good results. **Do not allow the wells to dry out.**

   Manual Wash Procedure:
   Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times.

6. Dispense 100µl of Conjugate (**Reagent 3)** into each well. Incubate the wells for **30** minutes at room temperature.

7. After 30 minutes, discard the well contents and carefully wash the wells 4 times with wash buffer. Ensure that the wells are empty but do not allow to dry out.

8. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate (**Reagent 4)** into each well. Incubate the plate for **10** minutes.

9. Add 100µl of Stop Solution (**Reagent 5)** to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.

10. Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes. A 620nm filter may be used as a reference wavelength.

11. Quality control

Quality control data is supplied on the lot-specific QC certificate included in the kit.

The positive control is intended to monitor for substantial reagent failure.

Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD-values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

12. Interpretation of Results

Quantitative results

Plot the OD of each standard against its value and draw the best-fit curve through the points. Read the unknowns off this curve. The 50 IU/ml standard is set at the upper limit of the normal range. Values between 40 and 50 IU/ml are considered borderline. Values above 50 IU/ml indicate elevated levels of antibodies to dsDNA. Patients with confirmed SLE have values in excess of 50 IU/ml.

Each laboratory should establish its own normal range. Samples with values above 400 U/ml should be re-assayed at a higher dilution.

Qualitative results

The 50 IU/ml standard is set at the upper limit of the normal range. Values above 50 IU/ml indicate elevated levels of antibodies to dsDNA.

13. Limitations of the Procedure

1. The assay shows no significant cross-reaction with single-stranded DNA antibodies.
2. Since IgG antibodies are thought to be most clinically significant, this assays uses an IgG specific conjugate. IgM dsDNA antibodies may compete with IgG antibodies for antigen binding. If interference from IgM antibodies is suspected, re-assay the sample at a higher dilution. A sharp increase in the value for the sample indicates the presence of competing IgM antibodies. This new value will give a more accurate measure of the dsDNA IgG antibody activity in the sample.
3. A negative dsDNA IgG result does not rule out the presence of SLE.
4. A positive dsDNA IgG result only indicates the presence of antibodies to dsDNA and does not necessarily indicate the presence of SLE.
5. Results of this assay should be interpreted in the light of all other available clinical findings.

Method Summary

- Dilute sera 1:100 with sample diluent **(Reagent 1)**
- Dispense standards, the positive control and the diluted sample into the microplate wells
- Incubate for **30** minutes at room temperature.
- *Wash the wells three times*
- Dispense 100µl of conjugate **(Reagent 3)** into each well
- Incubate at room temperature for **30** minutes
- *Wash the wells four times*
- Add 100µl of TMB substrate **(Reagent 4)** to each well
- Incubate at room temperature for **10** minutes
- Add 100µl stop solution **(Reagent 5)** to each well
- Read the optical density at 450nm (single wavelength) or 450/620nm (dual wavelength).

Further reading

Isenberg, D A et al: *Arthritis Rheumatol* 27 132-138 (1984)
Felkamp TEM et al: *Ann Rheum Dis* 47, 740-746 (1988)
Halbert SP et al: *J Clin Lab Med* 97, 97-111 (1981)
Tan EM et al: *Arthritis Rheumatol* 25, 1271-1277 (1982)
Tan EM et al: *Adv Immunol* 33,167-240 (1982)
Swaak AJG et al: *Ann Rheum Dis* **41**, 388-395 (1982)