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User Manual

Catalog Number: EKC36331

Product Name: Mouse anti-double stranded DNA antibody (IgG) ELISA Kit

Intended Use: For **qualitative** determination of mouse anti-double stranded DNA antibody (IGG) concentrations in serum.

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

Manual Version: 202301V1

Storage:

Unopened kit	6 months when stored at 2 - 8°C.
Opened Kit	May be stored up to 1 month at 2 - 8°C. Keep it in sealed aluminum foil bag and avoid moisture.

The product manual may be updated as a result of continuous improvements.

Always refer to the hard copy manual included in the kit for your experiment.

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Kit Components

Reagents	Quantity
Assay plate (12 x 8 coated Microwells)	1(96 wells)
Negative Control	1 x 0.8 ml
Positive Control	1 x 0.8 ml
Sample Diluent	1 x 50 ml
HRP-conjugate	1 x 10 ml
Wash Buffer (25 x concentrate)	1 x 20 ml
Substrate A	1 x 5 ml
Substrate B	1 x 5 ml
Stop Solution	1 x 5 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

Working Principle

This assay adopts qualitative enzyme immunoassay technique. Antigen is pre-coated onto a microplate. Samples are pipetted into the wells, together with anti-mouse antibody (IgG) conjugated Horseradish Peroxidase (HRP). Antibody specific to the antigen will bind to the antigen. Followed by washing to remove unbound reagents, and then adding substrate solution and later color develops in proportion to the amount of mouse anti-double stranded DNA antibody (IgG) bound in the sample. At last, measure the intensity of color after stopping color development.

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Specificity

This assay has high sensitivity and excellent specificity. No significant cross-reactivity or interference between mouse anti-double stranded DNA antibody (IgG) and analogues was observed. Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between mouse anti-double stranded DNA antibody (IgG) and all the analogues, therefore, cross reaction may still exist.

Precision

Intra-assay Precision (Precision within an assay): CV%<15%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<15%

Three samples of known concentration were tested in twenty assays to assess.

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Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100ml and 500ml graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

Sample Collection & Storage

- **Serum** Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation at 1000 ×g for 15 mins. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

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Sample Preparation

Dilute the serum samples with Sample Diluent(1:1000) before test. The suggested 1000-fold dilution can be achieved by adding 5μl sample to 95μl of Sample Diluent first, then complete the 1000-fold dilution by adding 5μl of this solution to 245μl of Sample Diluent.

Notes:

1. Biomatik is only responsible for the kit itself, not for the samples consumed during the assay. The user need to calculate the possible amount of the samples to be used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 2 days may be stored at 2-8°C, otherwise, samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid contamination and loss of bioactivity.
3. Grossly hemolyzed samples are not suitable to use.
4. It would be necessary to run a preliminary experiment for validation, if the samples are not indicated in the manual.
5. Recommend to use fresh samples for the test. If you store samples for long time, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

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Reagents Preparation

- **Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials in the kit.**
- Bring all reagents to room temperature (18-25°C) before use for 30 mins.
- Distilled Water is recommended. Contaminated water or container for reagents preparation will affect the test result.

Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

Precautions

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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Assay Procedures

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended to assay all samples in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.

2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.

3. Set a Blank well without any solution.

4. Set three **Negative Control** wells, two **Positive Control** wells.

5. Add 100µl **Negative Control**、**Positive Control** or **Diluted Sample** per well. Cover with the adhesive strip. Incubate at 37°C for 30 mins.

6. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (300µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 mins, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

7. Add 100µl **HRP-conjugate** to each well (Not to the Blank well). Cover the plate with a new adhesive strip. Incubate at 37°C for 30 mins

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8. Repeat the aspiration/wash process for five times as in step 6.

9. Add 50µl **Substrate A** and 50ul **Substrate B** to each well. Incubate at 37°C for 10 mins. **Protect from light.**

10. Add 50µl **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.

11. Take blank well as zero, determine the optical density of each well within 10 mins, using a microplate reader set to 450 nm.

***Samples may require dilution. Please refer to Sample Preparation.**

Notes:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.

2. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 mins. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To

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avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.

4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

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5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 mins), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

6. TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light.

7. Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

Calculation of Results

For calculation the valence of mouse anti-double stranded DNA antibody(IgG), compare the sample well with control.

The negative control OD value should be less than 0.15, and the positive control OD value should be greater than 0.6.

If ODnegative < 0.1, calculate it as 0.1.

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A cutoff value was defined as the average Negative Control value plus 0.1.

While ODsample≥Cutoff Value: Positive

While ODsample < Cutoff Value: Negative