Creative Diagnostics®

Version 22-04/24

User's Manual

Mouse Anti-Nucleosome Antibody IgG ELISA Kit

REF **DEIA-BJ2361**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

For the quantitative determination of mouse anti-nucleosome antibody (IgG) concentrations in serum, plasma.

Principles of Testing

This assay employs the quantitative sandwich enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with antigens. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antigen and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain anti-nucleosome antibody (IgG), biotin-conjugated antigen and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of anti-nucleosome antibody (IgG) in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Reagents And Materials Provided

1. Assay plate 1 (96 wells)
2. Standard (Lyophilized) 2
3. Biotin-conjugate (100 × concentrate) 1 × 120 µL
4. HRP-avidin (100 × concentrate) 1 × 120 µL
5. Biotin-conjugate Diluent 1 × 15 mL
6. HRP-avidin Diluent 1 × 15 mL
7. Sample Diluent 1 × 50 ml
8. Wash Buffer (25 × concentrate) 1 × 20 mL
9. TMB Substrate 1 × 10 mL
10. Stop Solution 1 × 10 mL
11. Adhesive Strip (For 96 wells) 4
12. Instruction manual 1

Materials Required But Not Supplied

1. Microtiter plate reader fitted with appropriate filters (450 nm required with optional 620 nm reference filter)
2. Microtiter plate washer or wash bottle
3. 10, 50, 100, 200 and 1000 µl adjustable single channel micropipettes with disposable tips
4. 50-300µl multi-channel micropipette with disposable tips
5. Multichannel micropipette reagent reservoirs

6. Distilled water
7. Vortex mixer
8. Orbital shaker
9. Miscellaneous laboratory plastic and/or glass, if possible sterile

Storage

Store at 2 - 8°C. Do not use the kit beyond the expiration date.

Specimen Collection And Preparation

1. **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 for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
2. **Plasma** Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 ×g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 month) to avoid loss of bioactivity and contamination.
2. Grossly hemolyzed samples are not suitable for use in this assay.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
5. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
6. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
7. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
8. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

Note:

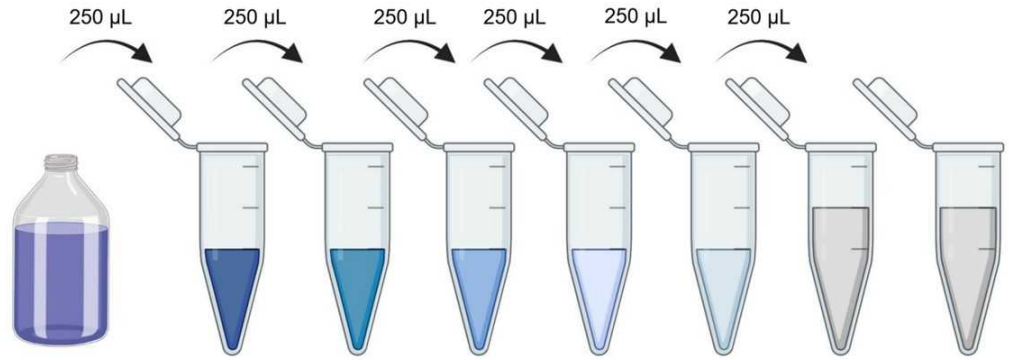
1. Kindly use graduated containers to prepare the reagent.Please don't prepare the reagent directly in the Diluent vials provided in the kit.
2. Bring all reagents to room temperature (18-25°C) before use for 30 min.
3. Prepare fresh standard for each assay. Use within 4 hours and discard after use.

4. Making serial dilution in the wells directly is not permitted.
5. Please carefully reconstitute Standards according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10μl for once pipetting.
6. Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

PROCEDURES:

1. **Biotin-conjugate (1×):** Centrifuge the vial before opening. Biotin-conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of Biotin-conjugate + 990 µl of Biotin-conjugate Diluent.
2. **HRP-avidin (1×):** Centrifuge the vial before opening. HRP-avidin requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP-avidin + 990 µl of HRP-avidin Diluent.
3. **Wash Buffer (1×):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (25 ×) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 ×).
4. Standard Centrifuge the standard vial at 6000-10000 rpm for 30s. Reconstitute the Standard with 1.0 ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 20 U/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   Pipette 250 µl of Sample Diluent into each tube (S0-S6). Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (20 U/ml). Sample Diluent serves as the zero standard (0 U/ml).



Tube	S7	S6	S5	S4	S3	S2	S1	S0
U/ml	20	10	5	2.5	1.25	0.625	0.312	0

Assay Procedure

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

1. Prepare all reagents, working standards, 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. Add 100 µL of standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at 37°C. A plate layout is provided to record standards and samples assayed.
4. Remove the liquid of each well, don't wash.
5. Add 100 µL of Biotin-conjugate (1×) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37°C. Biotin-conjugate (1×) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.)
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200 µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, 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 100 µL of HRP-avidin (1×) to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37°C.
8. Repeat the aspiration/wash process for five times as in step 6.
9. Add 90 µL of TMB Substrate to each well. Incubate for 15-30 minutes at 37°C. Protect from light.
10. Add 50 µL of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Note:

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 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To 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.
5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), 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

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the anti-nucleosome antibody (IgG) concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Precision

Intra-Assay: CV < 8%

Inter-Assay: CV < 10%

Detection Range

0.312 U/mL-20 U/mL

Sensitivity

0.078 U/mL

Specificity

This assay has high sensitivity and excellent specificity for detection of mouse anti-nucleosome antibody (IgG). No significant cross-reactivity or interference between mouse anti-nucleosome antibody (IgG) and analogues was observed.

Precautions

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

Limitations

1. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
2. The kit should not be used beyond the expiration date on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.
4. If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay.
5. Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
6. This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

Tel: 1-631-624-4882 (USA)
Tel: 44-161-818-6441 (Europe)
Fax: 1-631-938-8221
Email: info@creative-diagnostics.com