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Human Citrullinated Histone H3-DNA Complex (CitH3-DNA) ELISA Kit

Product Number: ELK12J1052

Shipping and Storage

Store at 2-8℃, store away from light and moisture.. Validity period: 6 months.

Component

Component	96-Well Configuration	48-Well Configuration
Pre-coated Assay Plate	8 wells×12 strips	8 wells×4 strips
Standards	0.3mL×6 pipes	0.3mL×6 pipes
Sample diluent	6mL	3mL
Detection Antibody-HRP	10mL	5mL
20×washing buffer	25mL	15mL
Substrate A	6mL	3mL
Substrate B	6mL	3mL
Stop solution	6mL	3mL
Plate Sealer	2 sheets	2 sheets

Note: The concentrations of standard samples (S0-S5) are 0、3、6、12、24、48μg/mL, respectively.

Description

This kit adopts the double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). Specimens, standards and HRP-labeled detection antibody are added sequentially into microwells pre-coated with antibody against CITH3-DNA complex (CITH3-DNA), followed by incubation and thorough washing. Color development is performed with TMB substrate. TMB is converted to blue under the catalysis of peroxidase, and finally turns yellow upon the addition of acid. The color intensity is positively correlated with CITH3-DNA complex (CITH3-DNA) in the sample. The absorbance (OD value) is measured with a microplate reader at a wavelength of 450 nm to calculate the sample concentration..

Specimen requirements

1. Serum: Avoid hemolysis. Use dry vacuum tubes without anticoagulant, or blood collection tubes with coagulant/gel separator. If blood collection tubes are unavailable, sterile and nuclease-free centrifuge tubes can be used. Keep the blood collection tube upright at room temperature (22–25°C) for 30–60 minutes to allow complete natural coagulation of blood. For tubes with coagulant, the standing time can be shortened to 15–30 minutes. Centrifuge at 3000–3500 rpm for 10–15 minutes at 4°C. Carefully aspirate the clear or pale yellow supernatant (serum) for testing.
2. Plasma: Avoid hemolysis. Use vacuum blood collection tubes containing anticoagulants (e.g., EDTA, sodium heparin or sodium citrate). If blood collection tubes are unavailable, add anticoagulant into sterile and nuclease-free centrifuge tubes. Gently invert and mix 5–8 times immediately after blood collection. No standing at room temperature (22–25°C); centrifuge promptly within 30 minutes after sampling. Centrifuge at 3000–3500 rpm for 10–15 minutes at 4°C. Carefully aspirate the clear or pale yellow supernatant (plasma) for testing.
3. Tissue:
   3.1. Animal tissue: After harvesting tissue (approximately 100 mg) from animals, rinse immediately with pre-cooled PBS to remove surface blood and impurities to prevent interference with subsequent assay results. Add protease inhibitor to PBS buffer at a ratio of 1:100. Add PBS buffer to the tissue at a ratio of 1 g : 9mL (the ratio may be adjusted appropriately

according to actual conditions, e.g., 1 g : 4mL, with proper documentation). Homogenize the tissue on ice using a mortar, or perform low-temperature homogenization with a tissue grinder. Alternatively, the tissue may be ground into powder with liquid nitrogen before adding PBS at the specified ratio, followed by lysis on ice for 30 minutes. Vortex vigorously for 15–30 seconds every 10 minutes during incubation for sufficient lysis. After grinding, transfer the homogenate into a new pre-cooled 1.5mL EP tube. Centrifuge at 10000 rpm for 15–20 minutes at 4°C, and collect the supernatant for testing.

3.2. Plant tissue: Collect fresh tissue, rinse thoroughly with deionized water, and blot surface moisture with filter paper. Weigh the required amount of tissue. Mince the tissue, then rapidly grind it into powder in a pre-cooled mortar with liquid nitrogen. Transfer the frozen powder into a centrifuge tube, add pre-cooled PBS at 4°C at the specified ratio (1 g : 9mL or 1 g : 4 mL; the ratio may be adjusted appropriately according to actual conditions with proper documentation), and homogenize on ice. Allow the homogenate to extract at 4°C for a period of time (typically 2 hours). Centrifuge at 3000 rpm for 15 minutes at 4°C to remove pigments as much as possible, then collect the supernatant for testing.

4. Cells:

4.1. Cell culture supernatant: Collect cell culture medium in sterile centrifuge tubes. Centrifuge at 4000 rpm for 15 minutes at 2~8°C to remove cell debris, particles and aggregates. Carefully aspirate the supernatant and transfer it into a new sterile centrifuge tube for testing.

4.2. Cell lysate: Discard cell culture supernatant, then gently wash the cells 1–2 times with pre-cooled PBS to remove residual culture medium. Add 150~250μL pre-cooled lysis buffer supplemented with protease inhibitor at a ratio of 1:100 per 10^6 cells. Lyse cells on ice or at 4°C for 15–30 minutes (repeated freeze-thaw or sonication is optional) with gentle shaking intermittently to facilitate lysis. Transfer the lysate to a centrifuge tube and centrifuge at 10000 rpm for 10 minutes at 4°C to remove intact cells, particles and aggregates. Carefully aspirate the supernatant for testing.

5. Secretions / excreta, etc.: For liquid samples, the general pretreatment procedure is centrifugation to remove impurities, precipitates, suspended matters, etc., and the supernatant is collected for testing. For solid samples (such as feces), add PBS (containing protease inhibitor) at an appropriate ratio (e.g., 1 g : 9mL) to lyse and release target analytes according to actual conditions. Multiple lysis methods are available, including homogenization, liquid nitrogen grinding, sonication, and repeated freeze-thaw (for cell samples). Centrifuge afterwards and collect the supernatant for testing.

Principles for Sample Aliquoting, Storage and Transportation

1. Principles for sample aliquoting and storage: Samples can be divided into small aliquots (e.g., 50~100μL per aliquot) and clearly labeled. One aliquot is used for immediate quantification, and the rest are stored at -80°C to avoid repeated freeze-thaw cycles (maximum 1–2 freeze-thaw cycles). After sample collection, samples to be tested within one week can be temporarily stored at 4°C; samples to be tested within one month can be temporarily stored at -20°C. For long-term storage, store samples at -80°C.
2. Transportation principles: Place sample tubes into a cryobox, then put the cryobox in the center of a foam box and fill the surrounding space with dry ice. Dry ice sublimes rapidly; prepare dry ice based on a consumption rate of approximately 5–10 kg per day.

Materials required but not supplied

1. Microplate reader (450 nm)
2. High-precision pipettors and tips: 0.5–10 μL, 2–20 μL, 20–200 μL, 200–1000 μL
3. 37°C incubator
4. Graduated cylinder: for preparation of 1× wash solution
5. PBS/TBS buffer, distilled or deionized water (for homogenization of solid samples such as tissues; to be supplied by user).

Reagent Preparation

Preparation of 1× Wash Solution: Dilute the concentrated wash solution with distilled water at a ratio of 1:20. For example, add 10 mL of concentrated wash solution into 190 mL of distilled water and mix thoroughly. (If crystals form in the 20× concentrated wash solution, heat at 37°C until all crystals dissolve before use.)

Washing method

1. Manual plate washing: Discard liquid in wells. Add 200–300μL of 1× wash solution to each well, incubate for 1 min, then remove all liquid from wells and tap dry on absorbent paper. Repeat the washing procedure for 5 times.
2. Automated plate washer: Dispense 200~300μL of 1× wash solution into each well, soak for 1 min, and wash for 5 cycles.

Preliminary Test Protocol

1. Purpose of preliminary test:

Perform serial dilution to identify the optimal sample dilution factor that brings the absorbance (OD value) of each target protein within the middle section (linear range) of the standard curve, so as to guarantee the accuracy and sensitivity of data in the formal assay.

2. Sample selection and dilution:

Select 1~2 samples from each experimental group and control group, generally the samples with the expected highest and lowest concentrations. Prepare serial dilutions for each sample at 2-fold, 5-fold and 10-fold dilutions. For samples expected to have low concentrations, undiluted stock, 2-fold and 5-fold dilutions may be adopted. Refer to the table below for operation:

Sample Concentration	Sample Name	Operating Procedure
Undiluted	N	Undiluted sample
2-fold dilution	1/2 N	50μL sample + 50μL diluent
5-fold dilution	1/5 N	20μL sample + 80μL diluent
10-fold dilution	1/10 N	10μL sample + 90μL diluent

3. Selection of optimal dilution factor:

Choose the dilution factor at which the sample OD value falls in the middle section of the standard curve (typically OD values between 0.8 and 2.0). This region features a steep curve slope, high sensitivity and the most accurate calculation.

Protocol

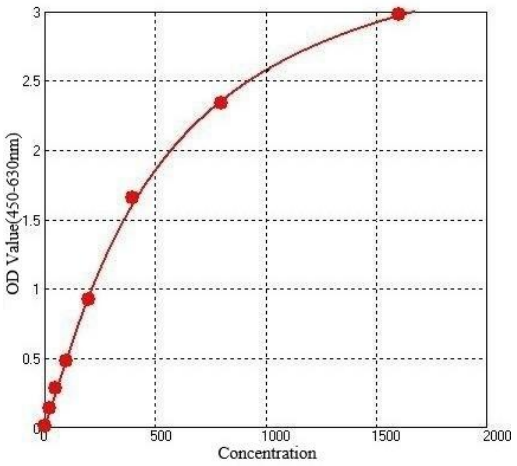
1. Reagent equilibration: Take the required strips from the foil pouch equilibrated to room temperature for 20 min. Seal the remaining strips in a ziplock bag with the supplied desiccant, protect from light, and store back at 4°C.
2. Adding standards: Set up standard wells and sample wells and keep proper records. It is recommended to mark the target name and strip sequence on the microplate to avoid confusion. Add 50μL of standards at different concentrations to each standard well.
3. Sample loading: For sample wells, add 10μL of test sample first, then add 40μL of sample diluent. The corresponding dilution factor (e.g., 5-fold) must be multiplied when calculating the final sample concentration. No addition for blank wells.
4. Enzyme addition: Add 100μL of enzyme conjugate (HRP-labeled detection antibody) to standard wells and sample wells except blank wells. Seal the reaction wells with plate sealer and incubate at 37°C in a water bath or incubator for 60 min.
5. Washing: Discard liquid and tap dry on absorbent paper. Fill each well with 250–300μL wash solution, incubate for 1 min, remove wash solution and tap dry on absorbent paper. Repeat the washing cycle 5 times (an automated plate washer may also be used).
6. Substrate addition: Add 50μL of chromogen A and 50 μL of chromogen B to each well, then develop color at 37°C in the dark for 15 min.
7. Stopping reaction: Add 50μL stop solution to each well. Measure the OD value of each well at 450nm within 15 min.
8. Note: 630nm serves as the reference wavelength to subtract non-specific background absorbance interference. Dual-wavelength reading mode is recommended. Subtract the OD value measured at 630 nm from the OD value measured at 450nm, and use the difference for result calculation, which can effectively eliminate non-specific absorbance interference caused by microplate bottom, liquid turbidity or sample matrix.

Result judgment

Export the data and calculate the average OD values of duplicate wells for standards and samples. Use ELISA Calc or other statistical software supporting 4-parameter curve fitting (the program can be obtained from the manufacturer). Take standard

concentration as the abscissa (X-axis) and absorbance OD value as the ordinate (Y-axis) to fit a 4-parameter logistic curve and obtain the equation. Substitute the sample OD value into the equation to calculate the sample concentration, then multiply by the dilution factor to get the actual sample concentration.

Concentration(ng/mL)	1600	800	400	200	100	50	25	0
OD Value(450nm)	3.018	2.385	1.702	0.963	0.529	0.325	0.183	0.062
OD Value(630nm)	0.042	0.046	0.045	0.038	0.045	0.043	0.046	0.045
Corrected OD Value	2.976	2.339	1.657	0.925	0.484	0.282	0.137	0.017



Test kit performance

1. Detection range: 0.75–48μg/mL.
2. Sensitivity: The minimum detectable concentration is less than 0.028μg/mL.
3. Precision: Intra-assay and inter-assay coefficients of variation are both less than 15%.
4. Specificity: No cross-reactivity with other soluble structural analogs.
5. Goodness of fit for standard curve: Coefficient of determination $R^2 \geq 0.99$ (closer to 1 is better).

Note

1. Storage and equilibration: The kit shall be stored at 2–8°C. After removal from refrigeration before use, allow the kit to equilibrate to room temperature of 20~25°C. Crystals may form in the concentrated wash solution taken out from the refrigerator, which is a normal phenomenon. Heat in a water bath to completely dissolve the crystals prior to use.
2. Storage of unused strips: For unused strips, remove them from the strip holder before reagent equilibration, place into a sealed bag with desiccant (supplied in the kit), seal tightly, and store refrigerated at 2~8°C protected from light.
3. Pipetting technique: Hold the pipette tip vertically and add samples without touching the well bottom. Perform gently to avoid bubble formation.
4. Washing procedure: Fill each well completely with wash solution, incubate for 30 seconds then aspirate, tap dry, and repeat for the specified number of times. Wipe off water stains and fingerprints on the plate bottom. Prevent microwells from remaining dry for extended periods throughout the procedure.
5. Substrate solution: Store protected from light. It should be colorless before use. Discard and do not use if it turns blue. Pipette the specified volume accurately; volume variation will directly affect assay repeatability.
6. Interference / cross-contamination: Change pipette tips between different samples. Do not interchange reagent caps to avoid cross-contamination and biased results. Avoid exposure to sodium azide (commonly used preservative) during the whole experiment, as sodium azide permanently inactivates HRP and severely interferes with assay results.
7. Avoid reagent mixing: Components from different batches or brands differ. Mixing may shift the standard curve and produce inaccurate results. Assays must be completed using reagents from the same batch.

8. BCA protein quantification: It is recommended to perform BCA quantification using the same sample supernatant, and normalize results to total protein content.
9. Post-assay handling: Stop solution is corrosive. Dispose of waste liquid as medical waste, disinfect bench surfaces, and discard gloves promptly. Remaining samples shall be properly disposed of in accordance with regulatory procedures.

Disclaimers

1. The reagent kit is for research purposes only and should not be used for clinical experiments or mouse experiments. Any consequences arising therefrom shall be borne by the experimenter, and our company shall not be held responsible.
2. Strictly follow the instructions for operation. If the experimenter violates the instructions, the consequences shall be borne by the experimenter.