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Mouse Urolithin A

Product Number: ELK17J0862

Shipping and Storage

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

Component

Component	96-Well Configuration	48-Well Configuration
Microplate	8 wells×12 strips	8 wells×6 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、30、60、120、240、480μmol/L, respectively.

Description

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

Specimen requirements

1. Serum: Use pyrogen and endotoxin-free tubes. Avoid any cellular stimulation during operation. After blood collection, centrifuge at 3000 rpm for 10 minutes to rapidly and carefully separate serum from red blood cells.
2. Plasma: Use EDTA, citrate or heparin for anticoagulation. Centrifuge at 3000 rpm for 30 minutes and collect the supernatant.
3. Cell culture supernatant: Centrifuge at 3000 rpm for 10 minutes to remove particles and aggregates.
4. Tissue homogenate: Homogenize tissue with an appropriate amount of normal saline. Centrifuge at 3000 rpm for 10 minutes and collect the supernatant.
5. Storage: If samples are not assayed immediately after collection, aliquot them for single use and store frozen at -20℃. Avoid repeated freeze-thaw cycles. Thaw at room temperature and ensure complete and uniform thawing of samples.

Materials required but not supplied

1. ELISA reader (450nm)
2. High precision sampler and nozzle: 0.5-10μL, 2-20μL, 20-200μL, 200-1000μL
3. 37℃ constant temperature box

Reagent Preparation

20×dilution of washing buffer: Dilute distilled water at a ratio of 1:20, which means adding 19 parts of distilled water to 1 part of 20×washing buffer.

Washing method

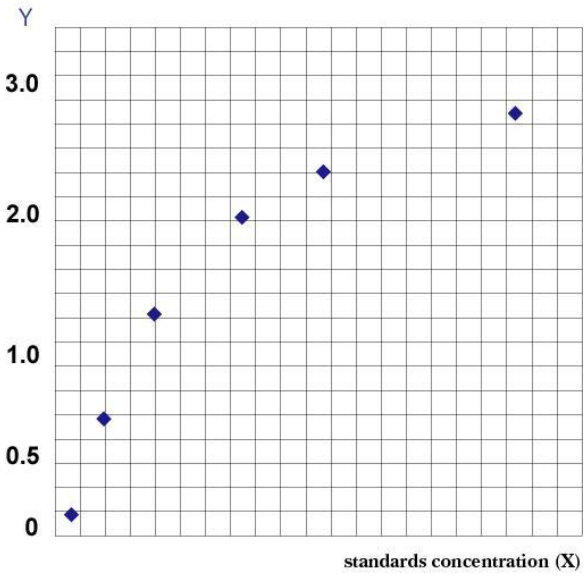
1. Manual board washing: Shake off the liquid in the holes, fill each hole with washing solution, let it stand for 1 minute, shake off the liquid in the holes, pat dry on absorbent paper, and wash the board 5 times in this way.
2. Automatic washing machine: Inject 350μL of washing solution into each well, soak for 1 minute, and wash the plate 5 times.

Protocol

1. Take the required microplate strips from the aluminum foil pouch which has been equilibrated to room temperature for 20 min. Seal the remaining strips in a zip-lock bag and store at 4°C.
2. Set wells for standards and samples. Add 50μL of standard solution at different concentrations to each standard well.
3. For sample wells, add 10μL of test sample first, then 40μL of sample diluent. Add nothing to blank wells.
4. Except blank wells, add 100μL of horseradish peroxidase (HRP) labeled detection antibody to each standard well and sample well. Seal the reaction wells with plate sealer and incubate at 37°C in water bath or incubator for 60 min.
5. Discard liquid and pat dry on absorbent paper. Fill each well with wash buffer, stand for 1 min, remove wash buffer and pat dry on absorbent paper. Repeat washing for 5 times (plate washer is also acceptable).
6. Add 50μL of Substrate A and 50μL of Substrate B to each well, incubate at 37°C in dark for 15 min.
7. Add 50μL of stop solution to each well. Measure OD value of each well at 450nm within 15 min.

Result judgment

Standard curve plotting: In an Excel worksheet, take the standard concentration as the abscissa and the corresponding OD value as the ordinate to plot the linear regression curve of standards, and calculate the concentration of each sample according to the curve equation.



Test kit performance

1. Accuracy: The correlation coefficient R value between the linear regression of the standard and the expected activity is ≥ 0.9900.
2. Sensitivity: The minimum detectable activity is less than 1.0μmol/L.
3. Specificity: No cross-reaction with other soluble structural analogs.
4. Repeatability: Both intra-plate and inter-plate coefficients of variation are less than 15%.

Note

1. The kit shall be stored at 2–8°C and equilibrated to room temperature for 20 minutes before use. Crystals may form in the concentrated wash solution taken out from the refrigerator, which is normal. Heat in a water bath until the crystals are completely

dissolved prior to use.

2. Unused microplate strips during the experiment should be immediately put back into the zip-lock bag and sealed for storage under low temperature and dry conditions.
3. Standard S0 with a concentration of 0 can be used as negative control or blank. Samples are diluted 5-fold during operation following the manual. Multiply the final reading by 5 to obtain the actual sample concentration.
4. Incubation shall be performed strictly in accordance with the incubation time, liquid volume and sequence specified in the manual. Thoroughly mix all liquid components before use..

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.