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Rat Interleukin 6 (IL-6) ELISA Kit

Product Number: RIL01

Shipping and Storage

1. 2-8℃, stored away from light and moisture
2. Validity period: 6 months

Component

Component	96T	48T
Microporous enzyme-linked immunosorbent assay plate	12 holes×8 strips	12 holes×4 strips
Standard Sample	0.3mL×6 tubes	0.3mL×6 tubes
Sample diluent	6mL	3mL
Detect antibody HRP	10mL	5mL
20× washing buffer solution	25mL	15mL
Substrate A	6mL	3mL
Substrate B	6mL	3mL
Stop solution	6mL	3mL
Microplate Sealers	2 sheets	2 sheets

Note: The concentrations of standard samples (S0-S5) are 0, 10, 20, 40, 80, and 160 pg/mL, respectively.

Description

The reagent kit adopts a double antibody one-step sandwich enzyme-linked immunosorbent assay (ELISA). Add the sample, standard, and HRP labeled detection antibody to the pre coated micropores with interleukin-6 (IL-6) antibody, incubate and thoroughly wash. Using substrate TMB for color development, TMB is converted to blue under the catalysis of peroxidase and to the final yellow under the action of acid. The depth of color is positively correlated with the level of interleukin-6 (IL-6) in the sample. Measure the absorbance (OD value) at a wavelength of 450nm using an enzyme-linked immunosorbent assay (ELISA) reader and calculate the sample concentration.

Specimen collection and storage

1. Serum: Use test tubes without pyrogen and endotoxin, avoid any cell irritation during the operation, collect blood, centrifuge at 3000 rpm for 10 minutes, and quickly and carefully separate serum and red blood cells.
2. Plasma: anticoagulant with EDTA, citrate or heparin. Centrifuge at 3000 rpm for 30 minutes and collect the supernatant.
3. Cell supernatant: Centrifuge at 3000 rpm for 10 minutes to remove particles and polymers.
4. Tissue homogenate: Crush the tissue by adding an appropriate amount of physiological saline. Centrifuge at 3000 rpm for 10 minutes and collect the supernatant.
5. Storage: If the sample is not tested in a timely manner after collection, please pack it according to a single dose and freeze it at -20℃ to avoid repeated freezing and thawing. Thaw it at room temperature and ensure that the sample is evenly filled and thawed.

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.

Note

For Research Use Only

1. The reagent kit should be stored at 2-8℃ and equilibrated at room temperature for 20 minutes before use. The concentrated washing solution taken out of the refrigerator will have crystals, which is a normal phenomenon. Heating in a water bath will completely dissolve the crystals before use.
2. The Flat noodles not used in the experiment should be immediately put back into the self sealing bag, sealed (low-temperature drying) and stored.
3. The S0 standard with a concentration of 0 can be considered as a negative control or blank; When operating according to the instructions, the sample has already been diluted 5 times, and the actual concentration of the sample is obtained by multiplying the final result by 5.
4. Strictly follow the time, liquid dosage, and sequence indicated in the instructions for incubation operation.
5. Shake all liquid components thoroughly before use.

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

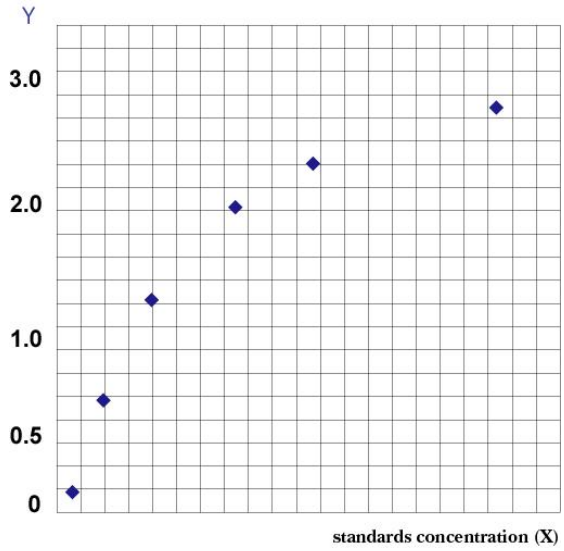
1. Manual board washing: Shake off the liquid in the holes, fill each hole with washing solution, let it stand for 1 minute, shake off the liquid in the holes, pat dry on absorbent paper, and wash the board 5 times in this way.
2. Automatic washing machine: Inject 350μL of washing solution into each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

Protocol

1. Take out the required Flat noodles from the aluminum foil bag after 20 min of room temperature balance, and seal the remaining Flat noodles with a self sealing bag and put it back at 4℃.
2. Set up negative and positive control wells and sample wells, and add 50μL of negative control and 50μL of positive control to each well; Blank hole not added.
3. Add 10μL of the test sample to the well, followed by 40μL of sample diluent;
4. Except for blank wells, 100μL of horseradish peroxidase (HRP) labeled detection antibody was added to each well of the standard and sample wells. The reaction wells were sealed with a plate membrane and incubated at 37℃ in a water bath or constant temperature incubator for 60 minutes.
5. Discard the liquid, pat dry on absorbent paper, fill each well with detergent, let it stand for 1 minute, shake off the detergent, pat dry on absorbent paper, repeat washing the board 5 times (or use a board washing machine).
6. Add 50μL of substrate A and B to each well, and incubate at 37℃ in the dark for 15 minutes.
7. Add 50μL of termination solution to each well and measure the OD value of each well at a wavelength of 450nm within 15 minutes.

Result judgment

Draw standard curve: In an Excel worksheet, use the standard concentration as the horizontal axis and the corresponding OD value as the vertical axis to draw a linear regression curve of the standard. Calculate the concentration values of each sample according to the curve equation.



Test kit performance

1. Accuracy: The correlation coefficient R value between standard linear regression and expected concentration is greater than or equal to 0.9900.
2. Sensitivity: The minimum detectable concentration is less than 1.0 pg/mL.
3. Detection range: 1-160 pg/mL.
4. Specificity: Does not cross react with other soluble structural analogues.
5. Repeatability: The coefficient of variation within and between plates is less than 15%.

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.