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Human Dityrosine ELISA Kit

Product Number: ELK018

Shipping and Storage

1. Reagent kit storage: 2-8℃.
2. Validity period: 6 months.

Component

Component	96T
30× concentrated wash buffer	20mL×1
Enzyme conjugate	6mL×1
Microplate (Coated)	12 wells×8 strips
Chromogen substrate A	6mL×1
Chromogen substrate B	6mL×1
Stop solution	6mL×1
Sample diluent	6mL×1
Standard S1 (400ng/L)	0.5mL×1
Standard S2 (200ng/L)	0.5mL×1
Standard S3 (100ng/L)	0.5mL×1
Standard S4 (50ng/L)	0.5mL×1
Standard S5 (25ng/L)	0.5mL×1

Description

This kit uses enzyme-linked immunosorbent assay to determine the level of Dityr in specimens. Coat a microplate with purified Dityr antibody to prepare a solid-phase antibody. Add Dityr and HRP labeled Dityr antigen to the micropores coated with the monoclonal antibody to compete for binding. After thorough washing, add substrate TMB for color development. The depth of sample color is negatively correlated with the content of Dityr in the sample. Measure the absorbance (OD value) at a wavelength of 450nm using an enzyme-linked immunosorbent assay (ELISA) reader, and calculate the content of Dityr in the sample using a standard curve.

Application

This kit is used to determine the content of Dityr in samples.

Testing Scope

10ng/L-500ng/L

Specimen requirements

1. Sample processing
   1.1. Water sample: After collection, it was repeatedly frozen and thawed three times at -20℃, and then filtered through glass fiber for future reference.
   1.2. Organization: The sample should be extracted with butanol: methanol: water (5:25:70 V: V: V) or according to relevant literature. The experiment should be conducted as soon as possible after extraction. If the experiment cannot be conducted immediately, the specimen can be stored at -20℃ for future reference.
2. Samples containing NaN3 cannot be detected because NaN3 inhibits the activity of horseradish peroxidase (HRP).

Protocol

1. Sample addition: Set up standard wells, blank wells (blank control wells do not contain samples or enzyme-linked immunosorbent assay reagents, all other steps are the same), and test sample wells. Add 50 microliters to the standard well on the enzyme-linked immunosorbent assay (ELISA) coated plate, then add 40μL of sample diluent to the test sample well, followed by 10μL of the test sample (with a final dilution of 5 times). Add the sample to the bottom of the enzyme-linked immunosorbent assay plate well, avoiding touching the well wall as much as possible, and gently shake and mix well.
2. Enzyme addition: Add 50μL of enzyme labeled reagent to each well, except for blank wells.
3. Incubation: Cover the plate with a sealing film and incubate at 37℃ for 60 minutes.
4. Solution preparation: Dilute 30 times the concentrated washing solution with distilled water and set aside for later use
5. Washing: Carefully remove the sealing film, discard the liquid, shake dry, fill each hole with detergent, let it stand for 30 seconds, then discard. Repeat this process 5 times and pat dry.
6. Color development: Add 50μL of color developing agent A50 to each well, then add 50μL of color developing agent B50, gently shake and mix, and develop color at 37℃ in the dark for 15 minutes
7. Termination: Add 50μL of termination solution to each well to terminate the reaction (at this point, the blue color turns yellow).
8. Measurement: Zero the blank well and measure the absorbance (OD value) of each well in sequence at a wavelength of 450nm. The measurement should be conducted within 15 minutes after adding the termination solution.

Calculation

Draw a standard curve on a coordinate paper with the concentration of the standard substance as the horizontal axis and the OD value as the vertical axis, and determine the corresponding concentration based on the OD value of the sample from the standard curve; Multiply by the dilution factor again; Alternatively, the regression equation of the standard curve can be calculated using the concentration and OD value of the standard substance. The OD value of the sample can be substituted into the equation to calculate the sample concentration, which is then multiplied by the dilution factor to obtain the actual concentration of the sample.

Note

1. The kit should be balanced at room temperature for 15-30 minutes before being taken out of the cold storage environment. If the enzyme coated plate is not used up after opening, the Flat noodles should be stored in a sealed bag.
2. Concentrated washing solution may precipitate crystals. When diluting, it can be dissolved by heating in a water bath, and washing does not affect the results.
3. Each step of sample addition should use a sampler and its accuracy should be regularly checked to avoid experimental errors. It is best to control the sample addition time within 5 minutes. If there are a large number of specimens, it is recommended to use a sampling gun for sample addition.
4. Please make a standard curve at the same time as each measurement, preferably with a double hole. If the content of the substance to be tested in the specimen is too high (the OD value of the sample is greater than the OD value of the first well of the standard well), please dilute the sample diluent by a certain multiple (n times) before measuring. When calculating, please multiply by the total dilution multiple (×n ×5) at the end.
5. The sealing film is only for one-time use to avoid cross contamination.
6. Please store the substrate away from light.
7. Strictly follow the instructions for operation, and the judgment of the test results must be based on the reading of the enzyme-linked immunosorbent assay (ELISA) reader
8. All samples, detergents, and various waste materials should be treated as infectious agents.
9. Components from different batch numbers of this reagent must not be mixed.