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Pyrroline-5-carboxylate (P5C) Elisa kit (Competition Methods)

Product Number: ELK11698

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
Sample diluent	6mL×1
Chromogen substrate A	6mL×1
Chromogen substrate B	6mL×1
Stop solution	6mL×1
Standard S1 (20ng/mL)	0.5mL×1
Standard S2 (10ng/mL)	0.5mL×1
Standard S3 (5ng/mL)	0.5mL×1
Standard S4 (2.5ng/mL)	0.5mL×1
Standard S5 (1.25ng/mL)	0.5mL×1
Standard diluent	1.5mL×1

Description

This kit employs the competitive enzyme-linked immunosorbent assay (ELISA) to determine the level of pyrroline-5-carboxylic acid (P5C) in specimens. Purified pyrroline-5-carboxylic acid (P5C) antibody is coated onto microplates to create solid-phase antibody. Pyrroline-5-carboxylic acid (P5C) and HRP-labeled pyrroline-5-carboxylic acid (P5C) antigen are added to the coated monoclonal antibody wells, where the two compete for binding. After thorough washing, a substrate TMB is added for color development. The intensity of the color is negatively correlated with the pyrroline-5-carboxylic acid (P5C) content of the sample. The absorbance (OD value) is measured at a wavelength of 450nm using an enzyme immunoassay reader, and the pyrroline-5-carboxylic acid (P5C) content of the sample is calculated via standard curve.

Application

This kit is used for the determination of pyrroline-5-carboxylic acid (P5C) levels in related liquid samples.

Testing Scope

0.4ng/mL - 25ng/mL

Specimen requirements

1. 1.Specimen treatment: (1) After collection, water samples are repeatedly frozen and thawed three times at -20℃ and then filtered through glass fiber for testing. (2) Tissue samples are extracted with butanol:methanol:water (5:25:70, V:V:V), or extracted according to the relevant literature; the assay should be carried out as soon as possible after extraction. If the assay cannot be carried out immediately, the specimen may be stored at -20℃ for later testing.
2. 2.Samples containing NaN3 cannot be detected because NaN3 inhibits the activity of horseradish peroxidase (HRP).

Protocol

1. Sample addition: Set up the standard wells, the blank wells (the blank control wells receive no sample and no enzyme conjugate; all other steps are identical) and the test sample wells. Add 50μL of standard to each standard well. For the test sample wells, first add 40μL of sample diluent, then add 10μL of test sample (final sample dilution = 5-fold). Dispense the sample to the bottom of each well, avoiding the well wall, and mix gently by swirling.
2. Enzyme addition: Add 50μL of enzyme conjugate to each well, except the blank wells.
3. Incubation: Seal the plate with the sealing film and incubate at 37℃ for 60 minutes.
4. Solution preparation: Dilute the 30× concentrated wash buffer 30-fold with distilled water and set aside for later use.
5. Washing: Carefully remove the sealing film, discard the liquid and shake dry. Fill each well with wash buffer, let it stand for 30 seconds, then discard. Repeat this 5 times and pat dry.
6. Color development: Add 50μL of chromogen substrate A to each well, then add 50μL of chromogen substrate B, mix gently with shaking, and develop the color at 37℃ in the dark for 15 minutes.
7. Termination: Add 50μL of stop solution to each well to stop the reaction (the color turns from blue to yellow immediately).
8. Measurement: Zero the reader against the blank well and measure the absorbance (OD value) of each well in sequence at a wavelength of 450nm. The measurement should be carried out within 15 minutes after adding the stop solution.

Calculation

Plot 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 read the corresponding concentration of each sample from the standard curve according to its OD value, then multiply by the dilution factor. Alternatively, calculate the linear regression equation of the standard curve from the concentrations and OD values of the standard substance, substitute the OD value of the sample into the equation to calculate the sample concentration, and multiply it by the dilution factor to obtain the actual concentration of the sample.

Note

1. The kit should be equilibrated at room temperature for 15-30 minutes after being taken out of cold storage before use. If the coated microplate is not used up after opening, the strips should be stored in a sealed bag.
2. Crystals may precipitate from the concentrated wash buffer. When diluting, they can be dissolved by warming in a water bath, and this does not affect the washing results.
3. Each dispensing step should be performed with a pipette, the accuracy of which should be checked regularly to avoid experimental errors. Dispensing should preferably be completed within 5 minutes; if there are many specimens, a multichannel pipette is recommended.
4. Please prepare a standard curve with every measurement, preferably in duplicate. If the content of the substance to be tested in a specimen is too high (the OD value of the sample is lower than the OD value of the first standard well), first dilute the sample with sample diluent by a certain factor (n-fold) before measurement, and at the end multiply by the total dilution factor (×n×5).
5. The sealing film is for single use only, to avoid cross contamination.
6. Please store the substrate away from light.
7. Strictly follow the instructions during operation; the judgment of the test results must be based on the readings of the microplate reader.
8. All samples, wash buffer and various waste materials should be treated as infectious materials.
9. Components from different batch numbers of this reagent must not be mixed.