Fumaric Acid (fumaric acid) Enzyme-Linked Immunosorbent Assay (ELISA) Kit Instruction Manual

F486219

This reagent kit is for research use only

Storage Conditions and Validity Period

Kit Storage: 2–8℃

Purpose of use

This reagent kit is used to determine the content of fumaric acid in the sample.

Experimental Principle

This kit applies the enzyme-linked immunosorbent assay (ELISA) competitive method to determine the level of fumaric acid in samples. Microplates are coated with purified fumaric acid antibodies to prepare solid-phase antibodies. Fumaric acid and horseradish peroxidase (HRP)-labeled fumaric acid antigens are added to the antibody-coated microwells, allowing them to compete for binding sites. After thorough washing, the substrate 3,3',5,5'-tetramethylbenzidine (TMB) is added for color development. The color intensity of the samples is negatively correlated with the fumaric acid content in the samples. The absorbance (OD value) is measured at a wavelength of 450 nm using a microplate reader, and the fumaric acid content in the samples is calculated via a standard curve.

Kit components

1	30× detergent	20ml×1bottle	8	Standard S1（200ng/L）	0.5ml×1bottle
2	enzyme-labeled reagent	6ml×1 bottle		Standard S2（100ng/L）	0.5ml×1bottle
3	ELISA plate	1well×8 strip		Standard S3（50ng/L）	0.5ml×1bottle
4	Chromogen Solution A	6ml×1bottle		Standard S4（25ng/L）	0.5ml×1bottle
5	Chromogen Solution B	6ml×1bottle		Standard S5（12.5ng/L）	0.5ml×1bottle
6	Termination Buffer	6ml×1bottle	9	Instruction Manual	1 copy
7	Dilution Buffer	6ml×1bottle	10	Sealing film	2 piece

Specimen Requirements

1．Specimen Preparation：

（1）**Water Samples** After collection, freeze and thaw the samples repeatedly at -20℃ for three cycles, then filter through glass fiber membranes before storage for testing.

（2）**Tissue Samples** Extract the samples with a mixture of butanol:methanol:water (5:25:70, *v/v/v*), or follow the extraction method described in relevant literature. Perform the experiment as soon as possible after extraction. If the experiment cannot be conducted immediately, store the specimens at -20℃ for future testing.Samples containing sodium azide (NaN_3) are **not suitable for detection**, as NaN_3 inhibits the activity of horseradish peroxidase (HRP).

Operating Procedures

1. Sample Loading： Set up standard wells, blank wells (no samples or enzyme-labeled reagents are added to the blank control wells; all other steps remain the same), and sample wells to be tested separately. Add 50 μl of standard solution to the standard wells of the pre-coated microplate. For the sample wells to be tested, first add 40 μl of sample diluent, then add 10 μl of the sample to be tested (the final dilution ratio of the sample is 1:5). Add the samples to the bottom of the microplate wells, avoid touching the well walls as much as possible, and gently shake to mix thoroughly.

2. Enzyme Addition： Add 50 μl of enzyme-labeled reagent to each well, except the blank wells.

3. Incubation Seal the microplate with a plate sealer and incubate at 37℃ for 60 minutes.

4. Solution Preparation： Dilute the 30× concentrated washing solution with distilled water at a ratio of 1:30, and set it aside for later use.

5. Washing： Carefully peel off the plate sealer, discard the liquid inside, and spin the plate to dry. Fill each well completely with the washing solution, let it stand for 30 seconds, then discard the solution. Repeat this washing process 5 times, and pat the plate dry finally.

6. Color Development： Add 50 μl of Chromogen A to each well first, then add 50 μl of Chromogen B. Shake gently to mix well, and incubate at 37℃ for 15 minutes in the dark.

7. Termination： Add 50 μl of stop solution to each well to terminate the reaction (the blue color will turn yellow immediately at this point).

8. Measurement： Zero the microplate reader with the blank wells, then measure the absorbance (OD value) of each well sequentially at a wavelength of 450 nm. The measurement should be completed within 15 minutes after adding the stop solution.

Calculation

Plot the standard curve on graph paper with the concentration of the standard as the x-axis and the OD value as the y-axis. Query the corresponding concentration from the standard curve according to the sample's OD value, then multiply it by the dilution factor.
Alternatively, calculate the linear regression equation of the standard curve using the concentration and OD value of the standard. Substitute the sample's OD value into the equation to obtain the sample concentration, then multiply it by the dilution factor to get the actual concentration of the sample.

Precautions

1. After removing the kit from refrigerated storage, allow it to equilibrate at room temperature for 1 hour before use. If the pre-coated microplate is not fully used after opening, place the unused plate strips in a sealed bag for storage.
2. Crystallization may occur in the concentrated washing solution. To dissolve the crystals, warm the solution in a water bath before dilution; this will not affect the test results.
3. A pipette should be used for sample addition in all steps, and its accuracy should be verified regularly to avoid experimental errors. The total time for a single round of sample addition is best controlled within 5 minutes. For a large number of samples, the use of a multi-channel pipette is recommended.
4. Prepare a standard curve for each assay, preferably with duplicate wells. If the concentration of the target substance in the sample is too high (the OD value of the sample is higher than that of the first standard well), dilute the sample by a certain factor (n-fold) with sample diluent before testing. When calculating the final concentration, multiply by the total dilution factor ($\times n \times 5$).
5. The plate sealer is for single-use only to prevent cross-contamination.
6. Store the substrate in the dark.
7. Follow the operating instructions strictly. The test results must be determined based on the readings from the microplate reader.
8. All samples, washing solutions, and waste materials should be disposed of as infectious substances.
9. Components from different batches of this kit must not be mixed.

Detection Range

4 ng/L – 300 ng/L

Specification

96 tests per kit