

KL-6 KIT

(Micro Cup-Type Enzyme Immunoassay Test Kit)

(For Research Use Only)

【Kit Contents】

Reagent	Components	Quantity	No.
Standard antigen	Tris buffer containing 0, 1, 2.5, 5, 10 and 20 U/mL of KL-6 antigen	0.3 mL/vial for all concentrations	1 vial per concentration
Sample diluent concentrate	Tris buffer containing bovine serum albumin	20 mL/vial	1 vial
Antibody-coated cup	Polystyrene microcup coated with solidified anti-KL-6 mouse monoclonal antibody	96 cups/package	1 package
Reaction solution	Tris buffer containing normal rabbit serum	15 mL/vial	1 vial
Enzyme-antibody conjugate concentrate	Solution containing horse radish peroxidase-labeled anti-KL-6 mouse monoclonal antibody	1.5 mL/vial	1 vial
Enzyme substrate	Oxydol (Japanese Pharmacopoeia)	0.5 mL/vial	1 vial
Chromogen	Lyophilized color former containing 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)	12 mL/vial (when dissolved)	3 vial
Stop-reaction solution	Sodium azide solution	15 mL/vial	1 vial
Wash solution concentrate	Physiological saline containing polyoxyethylene sorbitan monolaurate	10 mL/vial	1 vial

【Intended Use】

Determination of serum sialylated carbohydrate antigen KL-6

【Principles of the Method】

The principle of the assay is sandwich enzyme immunoassay (EIA), and the anti-KL-6 mouse monoclonal antibody (subsequently referred to as anti-KL-6 monoclonal antibody) is used both as a solid-phase antibody and as an enzyme-labeled antibody.

1. First reaction

Add serum sample to the cup which binds anti-KL-6 monoclonal antibody. KL-6 in the sample binds to the solid-phase antibody of the cup in proportion to the quantity added.

2. Second reaction

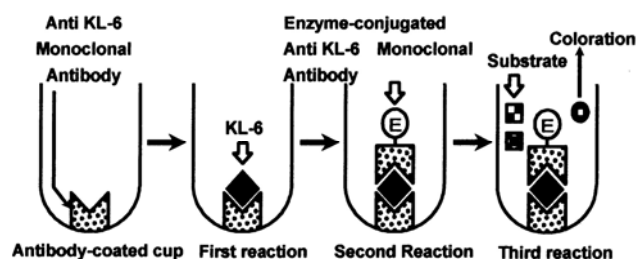
Remove the unreacted portion of the sample, and add the enzyme-labeled anti-KL-6 monoclonal antibody. A sandwich of solid-phase antibody, antigen (KL-6), and enzyme-labeled antibody is formed in proportion to the quantity of bound KL-6.

3. Third reaction

Remove the unreacted enzyme-labeled antibody, and add substrate to the cup. The substrate develops a color as it is decomposed by the bound enzyme-labeled antibody.

4. Measurement

Activity of the enzyme bound to the solid phase reflects the concentration of KL-6 in the sample. Measure the absorbance of the reacting solution and determine the concentration of KL-6 by comparing the absorbance the reacting solution with that of the standard antigen.

**【Procedure (Method and Materials)】****1. Reagent preparation****(1) Sample diluent preparation**

Dilute the sample diluent concentrate with purified water (1 part sample diluent concentrate with 9 parts purified water), and use the solution as the sample diluent.

When performing 96 tests, add 1 vial (20 mL) of the sample diluent concentrate to 180 mL of purified water. When performing 32 tests, add 6.5 mL of the sample diluent concentrate to 58.5 mL of purified water. The sample diluent remains stable for 4 weeks after preparation when stored at 2–10°C.

(2) Enzyme-antibody conjugate solution preparation

Dilute the enzyme-antibody conjugate concentrate with purified water (1 part enzyme-antibody conjugate concentrate with 9 parts purified water), and use the solution as the enzyme-antibody conjugate solution.

When making 96 tests, add 13.5 mL of the purified water to 1 vial (1.5 mL) of the enzyme-antibody conjugate concentrate. When making 32 tests, add 0.4 mL of the enzyme-antibody conjugate concentrate to 3.6 mL of purified water. The enzyme-antibody conjugate solution remains stable for 4 weeks after preparation when stored at 2–10°C.

(3) Wash solution preparation

Dilute the wash solution concentrate with physiological saline (1 part wash solution concentrate with 99 parts physiological saline), and use the solution as the wash solution.

When performing 96 tests, add 1 vial (10 mL) of the wash solution concentrate to 990 mL of physiological saline.

When performing 32 tests, add 3 mL of the wash solution concentrate to 297 mL of physiological saline. The wash solution remains stable for 4 weeks after preparation when stored at 2–10°C.

(4) Substrate solution preparation

Dissolve 1 vial of the chromogen in 12 mL of purified water, add 30 μL of the enzyme substrate, and use the solution as the substrate solution. Use the substrate solution immediately after preparation, and discard the left-over substrate solution.

(5) Reagents used as they are

Use the standard antigen, the antibody-coated cup, the reaction solution and the stop-reaction solution as they are.

2. Equipment

(1) Apparatus

Measuring cylinder, beaker, etc.

(2) Pipettes

Pipettes for measuring 10, 20, 30, 100, 500 μL and 1, 10 mL

(3) Test tubes

(4) Cup holders

Holders that hold antibody-coated cups in position

(5) Micromixer

(6) Light-shielding cover

(7) Cup washing machine

Well washer (e.g., minilab washer)

(8) Absorbance measuring apparatus

Plate reader (e.g., Sjeia Autoreader)

3. Procedure

(1) Sample dilution

Add 10 μL of a serum sample to 2 mL of the sample diluent (1 in 201 dilution).

(2) Place a necessary number of antibody-coated cups (subsequently referred to as cups) in the cup holders.

(3) Dispense 100 μL each of the reaction solution into the cups.

(4) Dispense 20 μL each of the standard antigen of differing concentration into 2 cups.

(5) Dispense 20 μL each of the diluted sample into 1 cup.

(6) After shaking, place the light-shielding cover, and let stand for 2 hours at 20–30°C.

(7) Discard the solution in the cups, wash in 3 changes of the wash solution, and remove the last traces of the wash solution in the cups.

(8) Dispense 100 μL each of the enzyme-antibody conjugate solution into the cups.

(9) Place the light-shielding cover and allow the solutions

to react for 1 hour at 20–30°C.

(10) Discard the solution in the cups, wash in 3 changes of the wash solution, and remove the last traces of the wash solution in the cups.

(11) Dispense 100 μL each of the substrate solution into the cups.

(12) Place the light-shielding cover and allow the reaction to take place for 30 min at 20–30°C.

(13) Dispense 100 μL each of the reaction-stop solution into the cups to stop the reaction.

(14) Read the absorbance of the reacting solution in the cup with a plate reader at a wavelength of 405 nm (λ_1). ($\lambda_2=492$ nm).

【Calculation Method of KL-6 Concentration】

1. Standard curve preparation

On logarithmic paper, plot the concentrations of each standard antigen (1, 2.5, 5, 10, 20 U/mL) as abscissae and the absorbance (mean) of each standard antigen solution minus the absorbance (mean) of 0 U/mL standard antigen as ordinates to obtain a standard curve.

2. Calculation of KL-6 concentration

Using the absorbance of a sample minus the absorbance (mean) of 0 U/mL standard antigen, determine the concentration of KL-6 from the standard curve, and calculate the concentration of KL-6 in the sample by multiplying the concentration of KL-6 by the dilution ratio (201).

Handling of serum samples outside the range of measurement

(1) For samples containing antigen at the concentration of more than 4020 U/mL, the concentration can be obtained by diluting them. (For example, a diluted sample is further diluted (1 in 11 dilution)).

(2) For samples containing antigen at the concentration of less than 201 U/mL, the concentration can be obtained in the range of 26–520 U/mL by changing the dilution ratio from 1 in 201 to 1 in 26.

【Specific Performance Characteristics】

1. Sensitivity

When 0 U/mL of the standard antigen is tested, the absorbance is not more than 0.08.

When 10 U/mL of the standard antigen solution is tested, the difference between its absorbance and the mean absorbance of 0 U/mL standard antigen is 0.45–0.85.

2. Specificity

When the control serum of the known concentration (350–450, 700–900, 2900–3500 U/mL) is tested, the measured value is 80–120% of the known concentration.

3. Reproducibility

When 2.5 U/mL and 10 U/mL of standard antigen are tested ($N=4$), the coefficient of variation (CV) is not

more than 10%.

4. Assay range

The range of serum measurement is 201–4020 U/mL when the test kit is used according to the specified procedure (1 in 201 dilution), and the range of the standard antigen measurement is 1–20 U/mL.

5. Dilution test

When the high concentration sample was diluted, the linearity was shown that the curve passed through the origin ranged to 4020 U/mL.

6. Recovery test

Recovery tests were performed with samples added with KL-6 antigen of known concentration. The recovery rate was 90–108%.

7. Effects of interfering substances

(1) Hemoglobin

No effect of hemoglobin was observed at the concentration of up to 1000 mg/dL.

(2) Bilirubin

No effect of free bilirubin and conjugated bilirubin was observed at the concentration of up to 50 mg/dL.

(3) Chyle

No effect of chyle was observed at turbidity of up to 500 (Holmadin index).

【Precautions】

1. General Precautions

- (1) Observe the notes on method and materials when making assay.
- (2) Kits of differing lot number should not be used in combination.
- (3) It has been confirmed that the standard antigens used in kits are negative for HBs antigen, HCV antibody, and HIV antibody. However, meticulous caution should be observed to avoid the risk of infection when handling KL-6 kits.

2. Precautions concerning samples

- (1) Use serum as a sample.
- (2) Do not use any samples that have been putrefied, denatured, deteriorated in improper storage.
- (3) Thoroughly mix the sample before using. Frozen samples may not be homogenous when thawed.
- (4) Samples may be contaminated with HIV, HTLV-1 or hepatitis virus. Observe caution against infection via wounds or oral infection.

3. Precautions

- (1) Acquire familiarity with the procedure before using. Temperature conditions should be strictly observed especially.
- (2) The weighing accuracy of pipettes and other apparatus is closely concerned with the precision of determination. Exercise due care when selecting

and operating apparatus. To avoid intercontamination between samples and reagents, do not use the same pipettes or tips for different samples and reagents.

- (3) Use the mean for duplicate assay every time the standard curve is plotted.
- (4) The reagents should be prepared before using as a rule. When several kits of the same lot number are to be used together at one time, the reagents from these kits should be transferred to one and the same container.
- (5) The antibody-coated cup should be used immediately after opening. Do not rub the pipettes against the insides of the cup.
- (6) Return the unused antibody-coated cups to the zippered aluminum foil bag and seal it completely.
- (7) When filling the cup with reagent and sample, exercise care so that its periphery is not soiled.
- (8) Avoid eye or hand contact with the enzyme substrate.

4. Disposition of Waste Matter and Used Apparatus

All samples, reagents, and equipment used in the test should be disposed of by any of the following methods.

- (1) Immersion in formalin solution (1 in 2,000) at 37°C for 72 hr or more.
- (2) Immersion in 2% glutaraldehyde solution for 1 hr or more.
- (3) Immersion in a 1 in 50–60 dilution of hypochlorite (12% sodium hypochlorite) for 1 hr or more.
- (4) If any of the above methods cannot be employed, autoclave at 121°C for at least 1 hr.

【Handling Precautions】

1. Precautions

The reagents should be prepared before using as a rule. Observe caution as suggested below when storing and reagent preparing.

- (1) Use the substrate solution immediately after preparation.
- (2) After preparation, store the diluted samples, the enzyme-antibody conjugate solution and the wash solution at 2–10°C and use them within 4 weeks of preparation. If a precipitate has occurred during storage, shake well before using.

2. Storage

Store at 2–10°C (do not freeze).

3. Effective period

The kit will be stable at 1 year after manufactured if recommended storage temperature is maintained. Do not use the kit beyond the expiration date stated on vial label and outer package.

4. Additional information

- (1) The reagents contain sodium azide as a preservative,

shown below. On disposal, flush with large volume of water.

The standard antigen : 0.1w/v%

The reaction solution : 0.1w/v%

The sample diluent concentrate : 1w/v%

The stop-reaction solution : 0.01w/v%

(2) Do not use the leftover wash solution for later use.

Treatment of wastes generated by this test should be done according to guidelines in accordance with the local laws and regulations.

【Packaging】

KL-6 KIT 1Box (96 tests)

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