

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 or 20 U/mL of KL-6 antigen	0.3 mL/vial for all concentrations	1 vial per concentration
Stock 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	A 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 assay is sandwich enzyme immunoassay (EIA) that uses anti-KL-6 mouse monoclonal antibody as a solid-phase antibody and a enzyme-labeled antibody a solid-phase antibody and the same anti-KL-6 mouse monoclonal antibody (subsequently referred to as anti-KL-6 monoclonal antibody) for labeling.

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 quantify added.

2. Second reaction

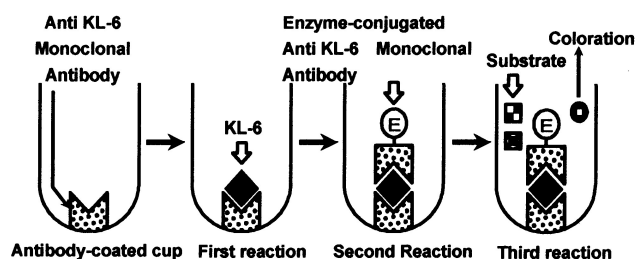
Remove the unreacted portion of the sample, and add the enzyme-labeled anti-KL-6 monoclonal antibody. A sandwich of solid-phase antibody, antigen (KL-6), and enzyme-labeled antibody is formed in proportion to the quantity of bound KL-6.

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 1:10 with purified water (1 part sample diluent concentrate with 9 parts purified water), and use this 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 concentrate preparation

Dilute the enzyme-antibody conjugate concentrate 1:10 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 3.6 mL of purified water. The Enzyme-antibody conjugate remains stable for 4 weeks after preparation when stored at 2-10°C.

(3) Wash solution preparation
Dilute the wash solution concentrate 1: 100 with physiological saline (1 part wash solution concentrate with 99 parts saline), and use this solution as the wash solution. When performing 96 tests, add 1 vial (10 mL) of the wash solution concentrate.

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) Enzyme substrate solution preparation.

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

(5) Reagents used as they are

Use the standard antigen, antibody-coated cups, reaction solution, and 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 10 mL

(3) Test tubes

(4) Cup holders

Holders that hold antibody-coated cups in position

(5) Micromixer

(6) Light-shielding covers

(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

To 2 mL of sample add 10 µL of serum sample (to obtain a 1 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 solution of differing concentration into 2 cups.

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

(6) After shaking and stirring, 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-labeled antibody 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 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 reaction stopping 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 solution (1, 2.5, 5, 10, 20 U/mL) as abscissae and the absorbance (mean) of each standard antigen solution minus the absorbance of 0 U/mL standard antigen solution 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 solution, 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 concentrations of more than 4020 U/mL, concentration, can be obtained by diluting them. (For example, a diluted sample is further diluted 1: 11).

(2) For samples containing antigen at concentrations of less than 201 U/mL, concentration can be obtained in the range of 26–5200 U/mL by changing the dilution ratio from 1:201 to 1: 26.

【Specific Performance Characteristics】

1. Sensitivity

When 0 U/mL of the standard antigen solution 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 to 0.85, under the conditions defined by manufacturer.

2. Specificity

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

3. Reproducibility (Within-run c.v. %)

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

5. Dilution test

When samples of high concentrations (4020 U/mL) were diluted, such linearity was shown that the curve passed through the origin.

6. Recovery test

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

7. Effects of interfering substances

(1) Hemoglobin

No effect of hemoglobin was observed at concentrations of up to 1000 mg/dl.

(2) Bilirubin

No effect of bilirubin, free or conjugated, was observed at concentrations 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 kit 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 1:50–60 dilution of hypochloride (12% sodium hypochloride) 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

This kit should be prepared before using as a rule. Observe caution as suggested below when storing opened and prepared kits.

- (1) Use the substrate solution immediately after preparation.
- (2) After preparation store the diluted samples, enzyme-labeled antibody solution, and 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 standard antigen and reaction solution contains 0.1w/v% and 2 mmol/L sodium azide as a preservative respectively. On disposal, flush with large volume of water.
- (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)

Distributed by : **SCETI K.K.**

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Manufactured by : **EIDIA Co., Ltd.**