

**KAMIYA BIOMEDICAL COMPANY**

# KL-6 ELISA

**For the quantitative determination of  
KL-6 in serum**

**Cat. No. KT-698**

**For Research Use Only.**

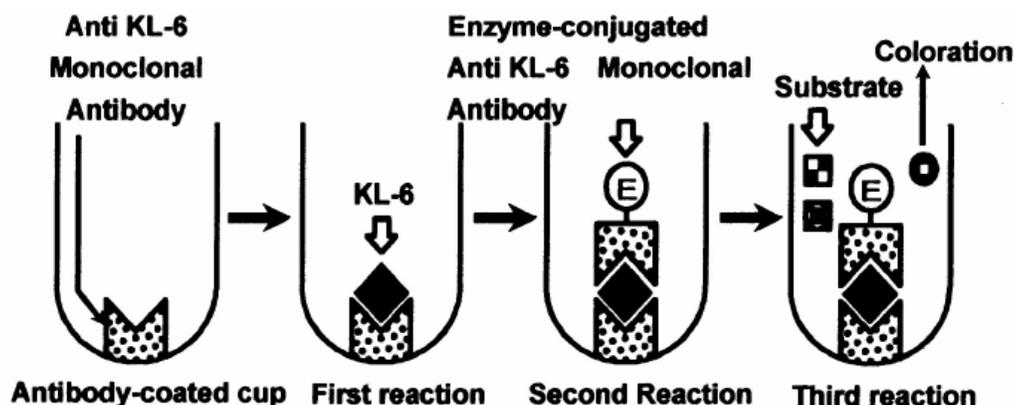
**PRODUCT INFORMATION****KL-6 ELISA**  
**Cat. No. KT-698****INTENDED USE**

The KL-6 ELISA is for the quantitative determination of sialylated carbohydrate antigen KL-6 in serum. For research use only. Not for use in diagnostics procedures.

**PRINCIPLE**

The principle of this assay is a sandwich ELISA that uses anti-KL-6 mouse monoclonal antibody as a solid-phase antibody and an enzyme labeled antibody for labeling.

1. **First Reaction**  
Add serum sample to the well which binds anti-KL-6 monoclonal antibody. KL-6 in the sample binds to the solid-phase antibody of the well 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 well. The substrate develops color as it is decomposed by the bonded 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 of the reacting solution with that of the calibrator antigen.

**COMPONENTS**

1. Calibrator Antigen: Tris buffer containing 0, 1, 2.5, 5, 10 and 20 U/mL of KL-6 antigen, 0.3 mL per vial
2. Diluent Concentrate: Tris buffer containing protein stabilizer, 20 mL
3. Antibody-Coated Plate: Polystyrene microplate coated with solidified anti-KL-6 mouse monoclonal antibody, 96 wells
4. Reaction Solution: Tris buffer containing normal rabbit serum, 15 mL
5. Enzyme-Antibody Conjugate Conc.: Solution containing HRP-labeled anti-KL-6 mouse monoclonal antibody, 1.5 mL
6. Enzyme Substrate: Oxydol, 0.5 mL
7. Chromogen: A lyophilized color former with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 3 x 12 mL
8. Stop Solution: Sodium azide solution, 15 mL
9. Wash Solution Concentrate: Physiological saline containing polyoxyethylene sorbitan monolaurate, 10 mL

## MATERIALS REQUIRED BUT NOT PROVIDED

- Measuring cylinder and beaker
- Pipettes for measuring 10, 20, 30, 100, 500  $\mu\text{L}$  and 1, 10 mL
- Test Tubes
- Plate holders
- Micromixer
- Light-shielding covers
- Plate washer
- Plate reader

## STORAGE

Store kit at 4°C. Do not freeze. The expiration date is printed on the box. Do not use the kit past the expiration date. After preparation, store the diluted samples, enzyme-labeled antibody solution, and wash solution at 4°C and use them within 4 weeks of preparation. If a precipitate has formed during storage, shake well before using.

## REAGENT PREPARATION:

1. 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 4°C.
2. Dilute the enzyme-antibody conjugate concentrate 1:10 with purified water (1 part enzyme-antibody conjugate with 9 parts purified water), and use the solution as the enzyme-antibody conjugate solution. When making 96 tests add 13.5 mL of 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 remains stable for 4 weeks after preparation when stored at 4°C.
3. 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 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 concentrate remains stable for 4 weeks after preparation when stored at 4°C.
4. Dissolve 1 vial of the chromogen in 12 mL of purified water, add 30  $\mu\text{L}$  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. Use the calibrator antigen, antibody coated wells, reaction solution and stop solution as provided.

## ASSAY PROTOCOL

1. To 2 mL of sample diluent add 10  $\mu\text{L}$  of serum sample (to obtain a 1 in 201 dilution).
2. Place the necessary number of antibody-coated wells in the well holders.
3. Dispense 100  $\mu\text{L}$  of the reaction solution into the wells.
4. Dispense 20  $\mu\text{L}$  each of the calibrator antigen solution of differing concentration into 2 wells.
5. Dispense 20  $\mu\text{L}$  each of the diluted sample into 1 well.
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 wells, wash in 3 changes of the wash solution and remove the last traces of the wash solution in the wells.
8. Dispense 100  $\mu\text{L}$  each of the enzyme-labeled antibody into the wells.
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 wells, wash in 3 changes of the wash solution and remove the last traces of wash solution in the wells.
11. Dispense 100  $\mu\text{L}$  each of substrate solution into the wells.
12. Place the light-shielding cover and allow the reaction to take place for 30 minutes at 20-30°C.

13. Dispense 100  $\mu$ L each of stop solution into the wells to stop the reaction.
14. Read the absorbance of the reacting solution in the wells with a plate reader at a wavelength of 405 nm ( $\lambda_1$ ). ( $\lambda_2 = 492$  nm).

## RESULTS

1. Calibration curve preparation  
On logarithmic paper, plot the concentrations of each calibrator antigen solution (1, 2.5, 5, 10, 20 U/mL) as abscissae and the absorbance (mean) of each calibrator antigen solution minus the absorbance of the 0 U/mL calibrator antigen solution as ordinates to obtain a calibration curve.
2. Calculation of KL-6 Concentration  
Using the absorbance of a sample minus the absorbance (mean) of the 0 U/mL calibrator antigen solution, determine the concentration of KL-6 from the calibration curve and calculate the concentration of KL-6 in the sample by multiplying the concentration of KL-6 by the dilution ratio (201).
3. Handling of serum samples outside the range of measurement
  - a. For samples containing antigen at concentrations of more than 4,020 U/mL, concentration can be obtained by diluting them. For example, a diluted sample is further diluted 1:11.
  - b. For samples containing antigen at concentrations less than 201 U/mL, concentration can be obtained in the range of 26-5,200 U/mL by changing the dilution ratio from 1:201 to 1:26.

## PERFORMANCE CHARACTERISTICS

1. Sensitivity  
When the 0 U/mL calibrator antigen solution is tested the absorbance is not more than 0.08.  
When the 10 U/mL calibrator antigen solution is tested, the difference between its absorbance and the mean absorbance of the 0 U/mL calibrator antigen solution is 0.45 – 0.85, under the conditions defined by the manufacturer.
2. Specificity  
When control serum of known concentration (350-450, 700-900, 2,900-3,500 U/mL) is tested, the measured value is 80-120% of the known concentration, under the conditions defined by the manufacturer.
3. Reproducibility  
When the 2.5 U/mL and the 10 U/mL calibrator antigen are tested (N=4) the coefficient of variation is not more than 10%.
4. Assay Range  
The range of serum measurement is 201-4,020 U/mL and the range of calibrator antigen measurement is 1-20 U/mL when this kit is used according to the specified procedure (201 dilution).
5. Dilution Test  
When samples of high concentrations (4,020 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 recovery was high, at 90-108%.
7. Effects of interfering substances
  - a. Hemoglobin  
No effect of hemoglobin was observed at concentrations of up to 1,000 mg/dL.
  - b. Bilirubin  
No effect of bilirubin, free or conjugated, was observed at concentrations of up to 50 mg/dL.
  - c. Chyle  
No effect of chyle was observed at turbidity of up to 500 (Holmadin index).

## PRECAUTIONS

1. This kit is for research use only. Not for use in diagnostic procedures.
2. Observe the notes on method and materials when performing the assay.
3. It has been confirmed that the calibrator antigens used in this kit 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.
4. Kits with different lot numbers should not be used in combination.
5. Only use serum as a sample

6. Do not use any samples that have putrefied, denatured, or deteriorated in improper storage.
7. Thoroughly mix the sample before using. Frozen samples may not be homogenous when thawed.
8. Samples may be contaminated with HIV, HTLV-1 or hepatitis virus. Observe caution against infection via wounds or oral infection.
9. Acquire familiarity with the procedure before using the kit. Temperature conditions must be strictly followed.
10. The accuracy of pipettes and other apparatus is closely concerned with the precision of determination; Exercise due care when selecting and operating apparatus. To avoid contamination between samples and reagents, do not use the same pipettes or tips for different samples and reagents.
11. Use the mean for duplicate assay every time the calibration curve is plotted.
12. If several kits of the same lot are to be used together at one time, the reagents from these kits should be transferred to one container.
13. The antibody-coated wells should be used immediately after opening. Do not rub the pipettes against the inside of the wells.
14. Return unused antibody coated wells to the zippered aluminum foil bag and seal it completely.
15. When filling a well with reagent and sample, exercise care so that its periphery is not soiled.
16. Avoid hand or eye contact with the enzyme substrate.
17. The calibrator antigen, sample diluent concentrate, stop solution and the reaction solution contain 0.1w/v% sodium azide as a preservative. On disposal, flush with large volume of water.
18. All samples, reagents, and equipment used in this test should be disposed of using one of the following methods:
  - a. Immersion in 2% glutaraldehyde solution for 1 hour or more.
  - b. Immersion in formalin solution (1:2,000) at 37 °C for 72 hours or more.
  - c. Immersion in a 1:50-60 dilution of hypochloride (12%) sodium hypochloride for 1 hour or more.
  - d. If none of the above can be completed, autoclave at 121 °C for at least 1 hour.
19. Use the substrate solution immediately after preparation.
20. 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.

**FOR RESEARCH USE ONLY**

**KAMIYA BIOMEDICAL COMPANY**

12779 Gateway Drive, Seattle, WA 98168  
Tel: (206) 575-8068 Fax: (206) 575-8094  
Email: LifeScience@k-assay.com  
www.k-assay.com