KAMIYA BIOMEDICAL COMPANY

Mouse IL-6 ELISA

For the quantitative determination of IL-6 in mouse serum or tissue culture supernatants

Cat. No. KT-669

For Research Use Only.

PRODUCT INFORMATION

Mouse IL-6 ELISA
Cat. No. KT-669

PRODUCT
The ***K-ASSAY***® Mouse IL-6 (Interleukin-6) ELISA is an enzyme immunoassay for the quantitative determination of IL-6 in mouse serum or tissue culture supernatants. For research use only.

INTRODUCTION
Interleukin-6 is a multifunctional cytokine that plays a key role in the acute phase response. Following tissue injury or infection, IL-6 is released from leukocytes, fibroblasts and/or endothelial cells. It subsequently triggers the hepatic synthesis of acute phase proteins that, depending on species, may include C-reactive protein, serum amyloid-A, alpha-1-acid glycoprotein, etc. Measurement of IL-6 in tissue culture supernatants or serum provides a convenient early stage biomarker of the inflammatory process. In house studies demonstrated that IL-6 peaked 4 hours after LPS challenge and returned to baseline values within 24h.

PRINCIPLE
The mouse IL-6 ELISA uses anti-mouse IL-6 antibodies for solid phase (microtiter wells) immobilization and biotinylated anti-mouse IL-6 antibodies for detection. Test samples, diluted as necessary, are incubated in the microtiter wells for 2 hours. The microtiter wells are subsequently washed, and biotinylated anti-mouse IL-6 is added and incubated for 1 hour. This results in mouse IL-6 molecules being sandwiched between the immobilization and detection antibodies. After washing the wells, horseradish peroxidase (HRP)-labeled avidin is added and incubated for 30 minutes. The HRP-avidin tightly binds to the biotinylated antibody. The wells are then washed to remove unbound HRP-labeled avidin, and TMB Reagent (an HRP substrate) is added and incubated for 20 minutes. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of mouse IL-6 is proportional to the optical density of the test sample and is derived from a calibration curve.

COMPONENTS
- Anti-mouse IL-6 coated microtiter plate (96 wells provided as 12 detachable strips of 8)
- Reference IL-6 stock (lyophilized), 2 vials
- 20X Wash Buffer, 50 mL
- Diluent, 2 x 50 mL
- TMB Reagent, 11 mL
- Stop Solution (1N HCl), 11 mL
- 2X Biotinylated mouse IL-6 detection antibody, 6 mL
- 2X Avidin-HRP conjugate, 6 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Precision pipettes and tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Graph paper (PC graphing software is preferable)
- Polypropylene or glass tubes
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an OD range of 0-4 OD
- Microplate incubator/shaker with an approximate mixing speed of 150 rpm
- Microplate washer

WASH SOLUTION PREPARATION

The wash solution is provided as a 20X stock. Prior to use dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water.

CALIBRATOR PREPARATION

1. The mouse IL-6 calibrator is provided as a lyophilized stock. Add the volume of diluent indicated on the vial label and mix gently until dissolved. This provides the 500 pg/mL working calibrator ***(the reconstituted calibrator remains stable for at least 7 days at 4°C but should be aliquoted and frozen at -20°C after reconstitution if use beyond this time is intended).***
2. Label 7 polypropylene or glass tubes as 250, 125, 62.5, 31.25, 15.63, 7.81 and 0 pg/mL, and dispense 250 µL of diluent into each tube.
3. Prepare the 250 pg/mL calibrator by diluting and mixing 250 µL of the 500 pg/mL calibrator with 250 µL of diluent in the tube labeled 250 pg/mL.
4. Similarly prepare the 125, 62.5, 31.25, 15.63, and 7.81 pg/mL calibrators by serial dilution.

SAMPLE PREPARATION

Our studies indicate that serum, or tissue culture supernatants containing 10% FBS, may be used undiluted. If dilution is necessary, use the diluent provided with the kit. We recommend that samples not tested immediately should be aliquoted and frozen at -80°C. Avoid repeated freeze-thaws.

DETECTION ANTIBODY PREPARATION

For each 8-well strip used in the assay, mix 0.5 mL of 2X mouse IL-6 detection antibody with 0.5 mL of diluent. **Prepare 5-10 minutes prior to use.**

AVIDIN-HRP CONJUGATE PREPARATION

For each 8-well strip used in the assay, mix 0.5 mL of 2X avidin-HRP conjugate with 0.5 mL of diluent. **Prepare 5-10 minutes prior to use.**

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and diluted samples into the wells (we recommend that calibrators and samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at 25°C for 2 hours.
4. Using a plate washer, wash the microtiter wells 4 times with 1x wash solution (350 µL per well per wash). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto paper towels to remove all residual droplets.
6. Add 100 µL of diluted biotinylated detection antibody into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at 25°C for 1 hour.
8. Wash as detailed in 4 and 5 above.
9. Add 100 µL of diluted avidin-HRP conjugate into each well.
10. Incubate on an orbital micro-plate shaker at 100-150 rpm at 25°C for 30 minutes
11. Wash the wells 6 times as detailed in 4-5 above.
12. Dispense 100 µL of TMB Reagent into each well.
13. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
14. Stop the reaction by adding 100 µL of Stop Solution to each well.
15. Gently mix if necessary. It is important to make sure that all the blue color changes to yellow.
16. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS

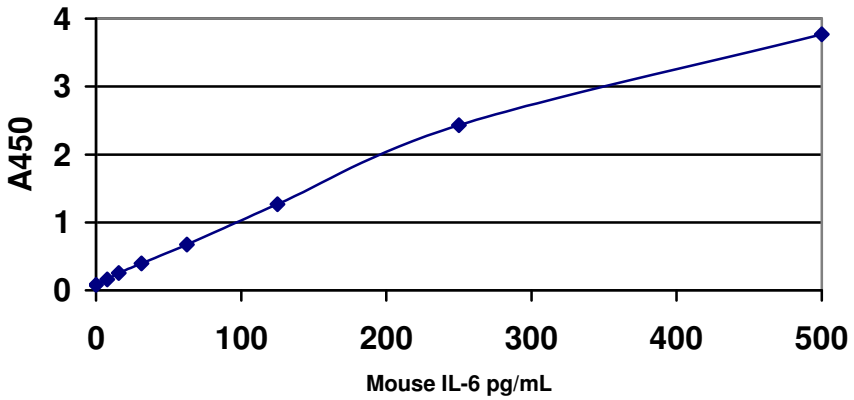
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in pg/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of mouse IL-6 in pg/mL from the calibration curve.
4. Multiply the derived concentration by the dilution factor, if applicable, to determine the actual concentration of mouse IL-6 n the original sample.

5. If available, PC graphing software should be used for the above steps. We find that a second order polynomial model usually provides a good fit for the calibration curve.
6. If the OD_{450} values of the sample fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density readings at 450 nm on the Y axis against mouse IL-6 concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and calibration curve in each experiment.

Mouse IL-6 (pg/mL)	Absorbance (450 nm)
500	3.771
250	2.432
125	1.268
62.5	0.673
31.25	0.395
15.63	0.253
7.81	0.161
0	0.080



STORAGE

Upon receipt, the lyophilized IL-6 stock should be stored at or below -20℃. The remainder of the kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date provided that the components are stored as described above.

GENERAL INSTRUCTIONS

All reagents should be allowed to reach room temperature (18-25℃) before use.

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