# Tribo™ Mouse IL-6 ELISA Kit (Catalog# TBS3040)

## DESCRIPTION

Interleukin-6 (IL-6) is a multifunctional proinflammatory cytokine which regulates immune response, acute-phase reactions, and hematopoiesis. IL-6 is a pleiotropic cytokine produced by a variety of cells such as hepatocytes, B and T lymphocytes, neurons, tumor cells, and multi-potent hematopoietic cells that acts on a wide range of tissues. It plays a central role in the production of a variety of pathological conditions including inflammatory responses, sepsis, rheumatoid arthritis, allergy, trauma, pain, neurodegenerative diseases, and several other autoimmune diseases.

The Tribo™ Mouse IL-6 ELISA Kit is designed to detect mouse IL-6 levels quantitatively in different tissue including skin, muscle, neural, serum, and other biological samples. The plate is coated with murine IL-6 monoclonal antibody and any IL-6 present in the sample or standard will be captured. A biotin-conjugated anti-murine IL-6 antibody is added along with streptavidin-horseradish peroxidase (HRP) accompanied by substrate. The reaction exhibits a blue color in direct proportion to the amount of IL-6 in the initial sample. The reaction can be terminated by the addition of a stop solution, and absorbance is measured at 450nm. The concentration of IL-6 in the samples is calculated opposite to the standard curve.

## APPLICATIONS

**Direct Assays:** quantitative IL-6 in different tissues.

## KIT CONTENTS

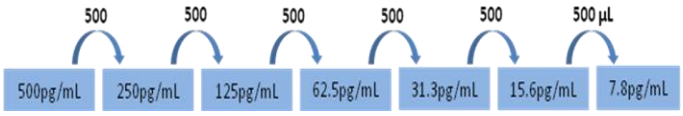
| Reagents | Quantity |
|---|---|
| Precoated 96-well Plate (8x 12 strips) | 1 plate |
| Standard | 1x 200µL |
| Detection A | 1x 100µL |
| Detection B | 1x 100µL |
| Sample Diluent (10x concentrate) | 1x 12ml |
| TMB Substrate Solution | 1x 12ml |
| Stop Solution | 1x 12ml |
| Wash Solution (20x concentrate) | 1x 50ml |
| Plate Sealers | 4 |

**Storage conditions: Store the kit at 4°C and standard at -20°C, respectively.**
**Shelf life: 12 months after receiving.**

## PROCEDURES

### Reagent Preparation:

1. Bring all reagents to room temperature (18-25˚C) before use. Briefly spin down all small tubes.
2. Wash Buffer: Add 50mL of 20x concentrated Wash Buffer into 950mL distilled water and mix thoroughly.
3. Reagent Diluent: Dilute 10mL of 10x concentrated Sample Diluent with 90mL of deionized water (1:10) before use.
4. Dilute Detection A: Add 100µL of the Detection A into 10mL of the Reagent Diluents for 96-well plate.
5. Dilute Detection B: Add 100µL of the Detection B into 10mL of the Reagent Diluents for 96-well plate.
6. Standard: Set up 7 points of IL-6 standard concentrations: 500, 250, 125, 62.5, 31.3, 15.6 and 7.8 pg/mL as shown in the diagram below.
7. Label 7 eppendoule tubes from 1-7. Add 900µL of the Reagent Diluents to the tube #1. Add 500µL of the Reagent Diluents to tubes #2-7.
8. Add 100µL of the IL-6 standard into tube #1 and vortex. This is Standard tube #1 with a concentration of 500pg/mL.
9. Standards #2-7 are then prepared by performing a 1:2 dilution of the preceding standard. Mix each tube thoroughly before the next transfer. For example, to make Standard #2, remove 500µL of Standard #1 and add it to tube #2 and vortex and so on. The Reagent Diluents serves as the zero standard (0 pg/ml).




### Test Procedures

1. Add 100µL of standards, blank and sample into plate in duplicate manner. Seal the plate and incubate for 2 hrs at room temperature.
2. Remove sealer and empty wells. Wash the plate 3 times with 150µL of Wash Buffer.
3. Add 100µL of the diluted Detection A to each well. Seal the plate and incubate for 2 hrs at room temperature.

4. Repeat washing step 2.
5. Add 100µL of the diluted Detection B to each well. Seal the plate and incubate for 20 minutes at RT.
6. Repeat washing Step 2.
7. Add 100µL of TMB Substrate Solution to each well. Incubate for 10-20 minutes at RT. Protect from light.
8. Add 50µL of Stop Solution to each well when the highest standard has developed a dark blue color. Gently tap the plate to ensure thoroughly mixing.
9. Read the optical density of each well immediately, using a microplate reader set to 450nm.

## CALCULATION

Average the duplicate readings for each standard, blank and samples then subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best-fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse IL-6 concentrations versus the log of the O.D. and the best-fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## MATERIALS REQUIRED, BUT NOT PROVIDED

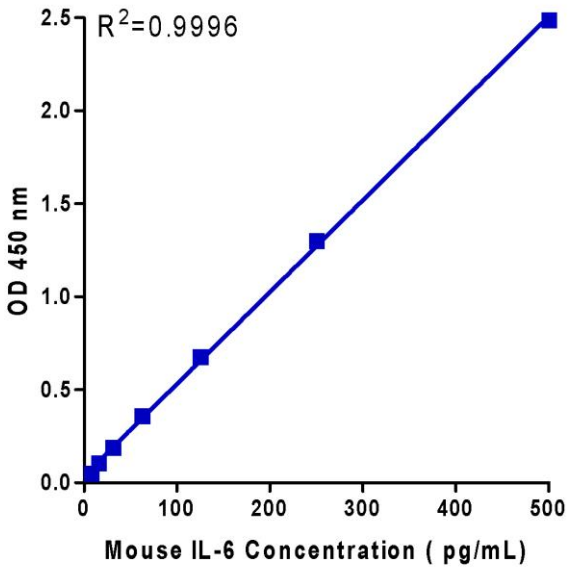
- Microplate reader
- Pipettes and Pipette tips
- Microtubes

## MAIN FEATURES

**Sensitivity:** The minimum detectable dose of mouse IL-6 is typically less than 8.0 pg/mL.

**Specificity:** This assay recognizes recombinant and natural mouse IL-6. No cross-reactivity with any of other cytokine is observed.

**Standard Curve of IL-6**




## REFERENCES

1. Van Snick J. IL-6: an overview. Ann. Rev. Immunol. 8: 253-278 (1990).
2. Chiu CP. et al. Multiple biological activities are expressed by a moue interleukin-6 cDNA clone isolated from bone marrow stromal cells. PNAS. 85: 7099-7103 (1988).

## RELATED PRODUCTS:

BSA Standard solution (Catalog# TBS5002)

Protein Cell Lysis Buffer (Catalog# TBS5001)

Protein Assay Kit (Catalog# TBS2005)

Mouse C5a ELISA (Catalog# TBS3001)

Mouse C3a ELISA (Catalog# TBS3020)

Mouse IL-1β ELISA (Catalog# TBS3030)

Mouse TNF-α ELISA I (Catalog# TBS3050)

TMB Substrate System (Catalog# TBS5021)