

For the quantitative determination concentrations of mouse IL-6 in cell culture supernatants, serum and plasma.

**INTRODUCTION**

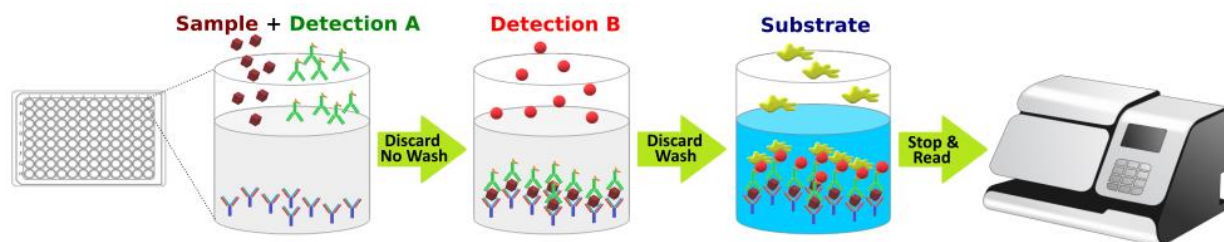
Interleukin-6 (IL-6) is a multifunctional proinflammatory cytokine which regulates immune response, acute-phase reactions, and hematopoiesis. It is a primary mediator in the inflammatory response, stimulating the production of acute phase proteins in the liver. IL-6 is produced by a variety of cells such as fibroblasts, hepatocytes, B and T lymphocytes, neurons, tumor cells, and multi-potent hematopoietic cells that act 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.

Tribioscience’s Fast Mouse IL-6 ELISA is designed to quantitatively detect mouse IL-6 levels in different tissues including skin, muscle, neural, serum, and other biological samples. The main feature is that **the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate, and fast. The measurement can be finished in 2 hours, with no need for 4-5 hours (Fig. 1). The detection range is from 15 to 1000 pg/mL.** The levels of mouse IL-6 samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural mouse IL-6 protein.

**PRINCIPLE OF THE ASSAY**

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (see Fig. 1). A monoclonal antibody specific for mouse IL-6 is pre-coated onto a microplate. Standards or samples and a biotin-conjugated detection antibody are pipetted into the wells and concurrently incubated to form a sandwich complex in one step. Simply aspirate each well without washing and directly add Streptavidin-HRP into the complex. Following a wash, an **ultra-sensitive TMB substrate solution** is added to the wells for color development. The color intensity is proportional to the amount of IL-6 bound in the initial step. The intensity of the color is measured by plate reading at 450 nm.

**Fig. 1**



**KIT CONTENT AND STORAGE CONDITIONS**

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Mouse IL-6 Microplate	TBS3040A	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse IL-6.	Return unused wells to the foil pouch. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Mouse IL-6 Standard	TBS3040B	30 µL of Recombinant mouse IL-6 protein (50 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3040C	190 µL of Biotin-mouse IL-6 antibody (12x).	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS3040D	300 µL of Streptavidin-HRP (50x).	
Assay Diluent	TBS3040E	15 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	15 mL of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date. The kit contains sufficient materials to run an ELISA on one 96 well plate.

**PRECAUTIONS**

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

**REAGENT PREPARATION**

**Bring all reagents to room temperature before use.**

**Wash Buffer:** Add 12 mL of Wash Buffer Concentrate (10X) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

**Detection A working solution preparation:** Add 175  $\mu\text{L}$  of **Detection A Stock** (Biotin-mouse –IL6 antibody) to 1925  $\mu\text{L}$  Assay Diluent to prepare Detection A working solution.

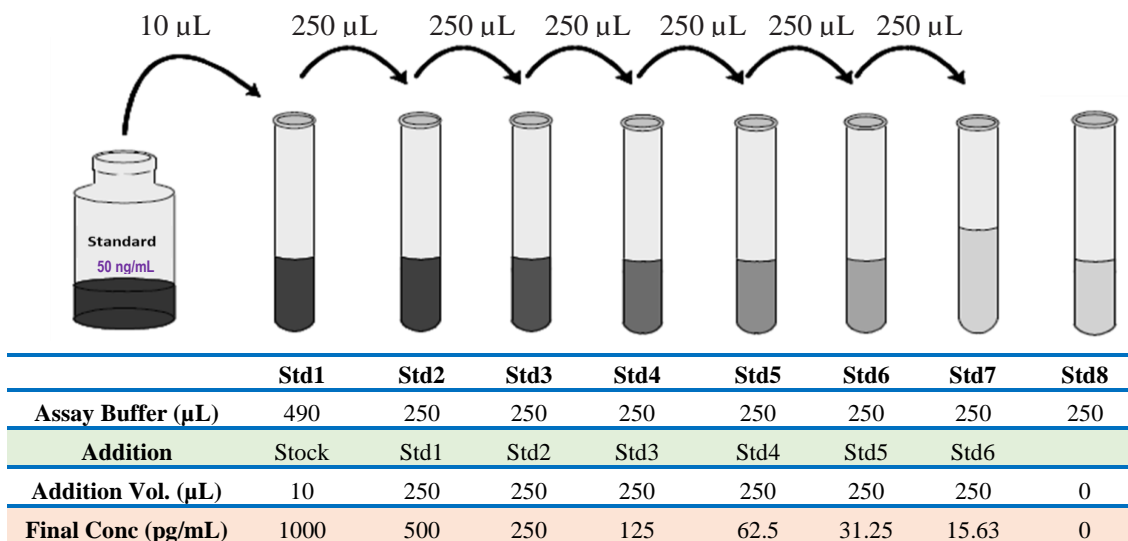
**Detection B working solution preparation:** Add 240  $\mu\text{L}$  of **Detection B** streptavidin-HRP to 12 mL Assay Diluent to prepare Detection B working solution.

**Mouse IL-6 Standard Preparation:**

Label test tubes as #1 through #8. Pipet 490  $\mu\text{L}$  of 1x Assay Diluent into tube #1, and 250  $\mu\text{L}$  into tubes #2 to #8 as Fig.2 diagram below.

1. Add 10  $\mu\text{L}$  of the Mouse IL-6 Standard stock solution (50 ng/mL) by dilution of 50X to tube #1 and mix.
2. Make 2x serial dilutions using the of 1000 pg/mL (tube #1) standard solution from tube #2 through #7 with sequential transfer of 250  $\mu\text{L}$  to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 1000, 500, 250, 125, 62.5, 31.25, and 15.63 pg/mL. Tube# 8 is blank (0 pg/mL).

**Fig.2 Diagram for Mouse IL-6 standard preparation**



**ASSAY PROCEDURE**

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.**

1. Add 80  $\mu\text{L}$  of standard, sample, or control per well.
2. Add 20  $\mu\text{L}$  of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 1 hour with shaking**.
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100  $\mu\text{L}$  of **Detection B working solution** to each well. Incubate at **RT for 0.5 hour with shaking**.
5. Aspirate each well, and wash for 3 times by filling each well with 300  $\mu\text{L}$  Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100  $\mu\text{L}$  of **TMB Substrate** to each well. Incubate **at RT for 10-20 minutes with shaking** (*Protect from*

- light*). The color becomes blue.
7. Add 50  $\mu$ L of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
  8. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

**CALCULATION OF RESULTS**

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve 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.

**TYPICAL DATA**

This standard curve ( $R^2=0.9998$ ) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

**SENSITIVITY**

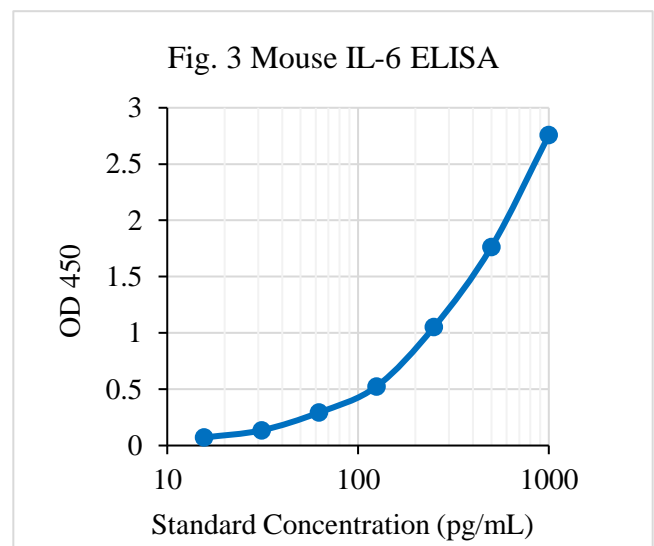
The minimum detectable dose (MDD) of mouse IL-6 is typically 10 pg/ml.  
The Intra-assay CV is 3.79% the Inter-assay CV is <10%.

**SPECIFICITY**

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

**RELATIVE PRODUCTS**

- TBS3030 Fast Mouse IL-1 $\beta$  ELISA
- TBS3031 Fast Mouse IL-2 ELISA
- TBS3032 Fast Mouse IL-4 ELISA
- TBS3044 Fast Mouse IL-10 ELISA
- TBS3047 Fast Mouse IL-12 p70 ELISA
- TBS3049 Fast Mouse IL-13 ELISA
- TBS3050 Fast Mouse TNF- $\alpha$  ELISA
- TBS3060 Fast Mouse KC ELISA
- TBS3070 Fast Mouse NGF ELISA
- TBS3079 Fast Mouse GM-CSF ELISA
- TBS3080 Fast Mouse G-CSF ELISA
- TBS3084 Fast Mouse IFN- $\gamma$  ELISA
- TBS3085 Fast Mouse TGF ELISA
- TBS3086 Fast Mouse MCPT-1 ELISA
- TBS3090 Fast Mouse IL-17AF ELISA
- TBS3091 Fast Mouse IL-19 ELISA
- TBS3092 Fast Mouse IL-21 ELISA
- TBS3093 Fast Mouse IL-22 ELISA
- TBS3094 Fast Mouse IL-23 ELISA
- TBS3095 Fast Mouse IL-27 ELISA
- TBS3096 Fast Mouse IL-28B ELISA
- TBS3097 Fast Mouse IL-33 ELISA



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