Tribioscience

Mouse IL-10 Fast ELISA (Catalog: TBS3044)

For the quantitative determination of mouse IL-10 concentrations in cell culture supernatants, serum, and tissues.

INTRODUCTION

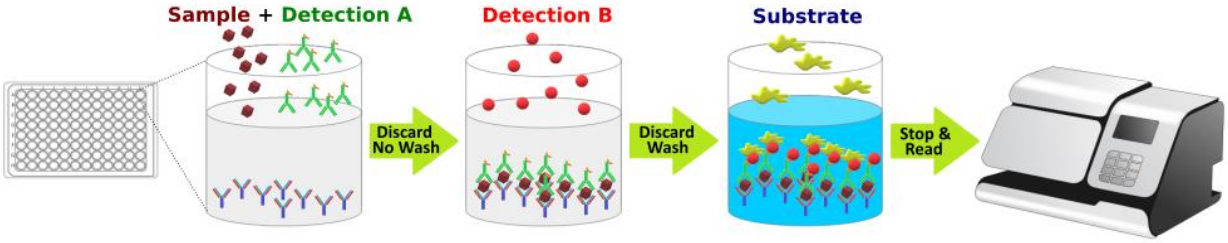
Interleukin-10 (IL-10) is a multifunctional cytokine and primarily produced by monocytes. It plays an important role as a regulator of lymphoid and myeloid cell function. This cytokine has various effects on immunoregulation and inflammation, blocking cytokine synthesis and several accessory cell functions of macrophages, T-cells, and NK cells. In addition, IL-10 participates in the regulation of proliferation and differentiation of B-cells, mast cells, and thymocytes. Murine IL-10 exhibits strong homology to human IL-10 and an open reading frame in the Epstein-Barr virus genome, BCRF1, which shares many of the cellular cytokine's biological activities and may therefore play a role in the host-virus interaction. Mutations in this gene have been associated with an increased susceptibility to HIV-1 infection and rheumatoid arthritis.

Tribioscience's Mouse IL-10 Fast ELISA kit is designed to quantitatively detect mouse IL-10 levels in tissues, serum, plasma, 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 (Fig. 1). The detection range is from 31 to 2000 pg/mL. The levels of mouse IL-10 samples are parallel to the standard curves obtained using the kit standards linearly. Therefore, the kit can be used to determine relative mass values for natural mouse IL-10 protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for mouse IL-10 was pre-coated onto a microplate. Standards and samples are pipetted into the wells, and then incubated with HRP-conjugated detection antibody specific for mouse IL-10. Following a wash to remove any unbound antibodies and samples, an ultra-sensitive TMB substrate solution is added to the wells for color development. The color intensity is in proportion to the bound amount in the initial step.The intensity of the color is measured by plate read at 450 nm.

Fig. 1



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Mouse IL-10 Microplate	TBS3044A	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-10.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Mouse IL-10 Standard	TBS3044B	20 µL of Recombinant mouse IL-10 (200 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	TBS3044C	2.1 mL of biotin-mouse IL-10 antibody.	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS3044D	200 µL of streptavidin HRP (80x).	
Assay Diluent	TBS3044E	25 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 mL of concentrated solution (10X)	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8℃. 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.

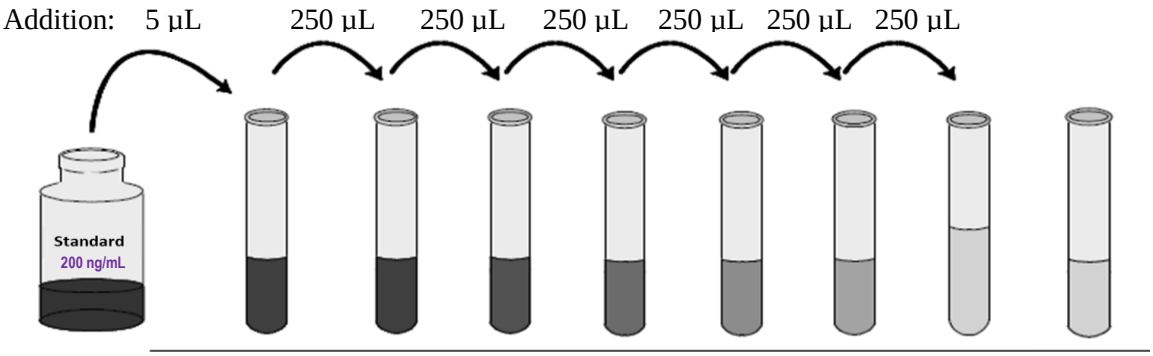
Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10X) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved).*

Detection B working solution preparation: Add 150 µL of **Detection B** streptavidin-HRP to 12 mL Assay Diluent (TBS3044E) to prepare Detrection B working solution.

Mouse IL-10 Standard Preparation: Label test tubes as #1 through #8. Pipet 495 µL of Assay Diluent into tube #1, and 250 µL into tubes #2 to #8 **as Fig.2 diagram below.**

1. Add 5 µL of the **Mouse IL-10** Standard stock solution (200 ng/mL) to tube #1 and mix.
2. Make 2x serial dilutions of the standard using the Tube#1(2000 pg/mL standard solution) from Tube #2 through #7 with sequential transfer of 250 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 2000, 1000, 500, 250, 125, 62.5 and 31.3 pg/mL. Tube# 8 is blank (0 pg/mL).

Fig.2 Diagram for Mouse IL-10 standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	495	250	250	250	250	250	250	250
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	5	250	250	250	250	250	250	0
Final Conc (pg/mL)	2000	1000	500	250	125	62.5	31.3	0

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 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours with gentle shaking.**
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B working solution** to each well. Incubate at **RT for 1 hour with gentle shaking**.
5. Aspirate each well, and wash 3 times by filling each well with 300 µL 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 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20 minutes with gentle shaking** (*Protect from light*). The color becomes blue.
7. Add 50 µL 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 542 nm or 570 nm. If wavelength correction is not available, subtract readings at 542 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 human 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=0.9997) 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 (MOD) of mouse IL-10 is typically 31 pg/mL.

The Intra-assay CV and the Inter-assay CV are <10%.

SPECIFICITY

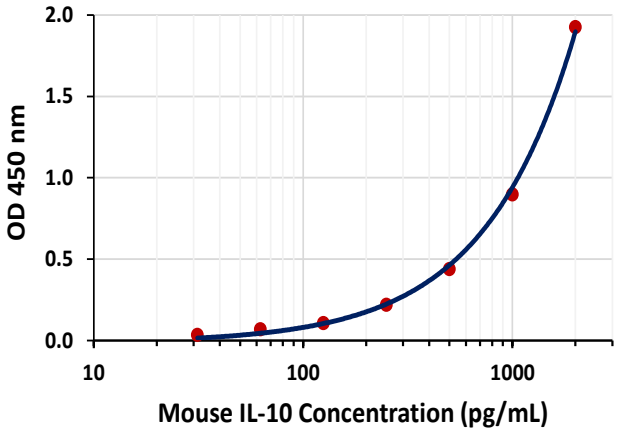
This assay recognizes natural and recombinant mouse IL-10.

No cross-reactivity with human, canine, feline and porcine IL-10.

RELATIVE PRODUCTS

TBS3030	Fast Mouse IL-1β ELISA
TBS3031	Fast Mouse IL-2 ELISA
TBS3032	Fast Mouse IL-4 ELISA
TBS3040	Fast Mouse IL-6 ELISA
TBS3047	Fast Mouse IL-12 p70 ELISA
TBS3049	Fast Mouse IL-13 ELISA
TBS3050	Fast Mouse TNF-α 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-γ 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
TBS3098	Fast Mouse Insulin ELISA

Fig.3 Mouse IL-10 Standard Curve



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