Mouse IL-10 ELISA Kit

Catalog No. EZMIL10

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

Introduction

Mouse IL-10 (Interleukin 10, B cell derived T cell growth factor, B-TCGF, Cytokine synthesis inhibitory factor CSIF, T-cell growth inhibitory factor, TGIF) was originally described as Cytokine Synthesis Inhibitory Factor (CSIF) due to its ability to inhibit cytokine production by TH1 clones. IL-10 is expressed in activated $CD8^+$ and $CD4^+$ T cells, activated monocytes, mast cells, and Ly-1 B cells. IL-10 shares over 80% sequence homology with the Epstein-Barr virus protein BCRFI that allows the virus to suppress the immune response. The functions of IL-10 include inhibition of macrophage-mediated cytokine synthesis, suppression of the delayed type hypersensitivity response, and stimulations of the TH2 cell response.

The EMD Millipore Mouse IL-10 ELISA Kit is a Sandwich Enzyme- Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a capture antibody. This kit is specifically designed for the accurate quantification of mouse IL-10 from cell culture supernatant, serum, plasma, and other biological fluids. This kit is analytically validated with ready-to-use reagents.

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Kit Components

- Mouse IL-10 Pre-Coated Plate (Part No. CS210111): One 96-well plate
- Mouse IL-10 Detection Antibody (Part No. CS210112): One 12 mL bottle
- Mouse IL-10 Standard (Part No. CS210113): One lyophilized vial
- Matrix C (Part No. CS210060): One lyophilized vial
- Avidin HRP D (Part No. CS210086): One 12 mL bottle
- Assay Buffer A (Part No. CS210062): One 25 mL bottle
- Wash Buffer (20X) (Part No. CS210053): One 50 mL bottle
- Substrate Solution F (Part No. CS210054): One 12 mL bottle
- Stop Solution (Part No. CS213422): One 12 mL bottle
- Plate Sealers (Part No. CS210056): One 4 pack

Materials Not Supplied

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage & Stability Information

Store kit and unopened components at 2℃ to 8℃

Stability: Kit components are stable for a minimum of 4 months from the date of receipt if stored and handled as described below.

Reagent Precautions

- **Safety Warnings and Precautions:** This kit is designed for research use only and is not recommended for internal use in mice or animals. All chemicals should be considered potentially hazardous and principles of good laboratory practice should be followed.
- This kit is not for use in diagnostic procedures.
- Caustic Material: Stop Solution. The stop solution contains acid which is harmful if swallowed or inhaled; avoid contact with skin and eyes; wash areas of contact immediately with water. **Caution: Eye, hand, face, and clothing protection should be worn when handling this material.**
- Substrate Solution F is harmful if inhaled or ingested. Additionally, avoid skin, eye or clothing contact with the substrate reagents
- The instructions provided have been designed to optimize the kit's performance. Deviation from the instructions may result in suboptimal performance of the kit and a failure to produce accurate data.

Specimen Collection and Handling

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantification.

Cell Culture Supernatant

If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70℃. Avoid repeated freeze -thaw cycles.

Serum

Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70℃. Avoid repeated freeze-thaw cycles.

Plasma

Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70℃. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation

- Note: All reagents should be diluted immediately prior to use.
- 20X Wash Buffer: The Wash Buffer provided is supplied as a 20X concentrate. Verify that it does not contain precipitate prior to diluting it to 1X. If necessary, warm to room temperature (20℃ to 25℃) prior to dilution. Dilute the conce ntrate to 1X strength using Milli-Q™ or deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water.
- Mouse IL-10 Standard: Reconstitute the lyophilized Mouse IL-10 Standard by adding the volume of Assay Buffer A indicated on the vial label to make the 20 ng/mL standard stock solution. Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.
- If serum or plasma samples will be assayed, reconstitute the lyophilized Matrix C by dispensing 2 mL of Milli-Q™ or deionized water into the vial and allow to sit at room temperature for 15 minutes, then vortex completely to mix.

Assay Protocol

1. Prepare the reagents as described in the Reagent Preparation section.
2. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
3. Identify the number of reaction wells needed and remove any unneeded strip wells (8 wells per strip) from the plate holder. To do so carefully turn the plate upside down and push the strips from the bottom out through the plate. Re-seal the extra strips in the resealable bag with desiccant and store in the refrigerator for future use.
4. Prepare 500 µL of the 4000 pg/mL top standard by diluting 100 µL of the standard stock solution in 400 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 4000 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the mouse IL-10 standard concentrations in the tubes are 4000 pg/mL, 2000 pg/mL, 1000 pg/mL, 500 pg/mL, 250 pg/ mL, 125 pg/mL, and 62.5 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).

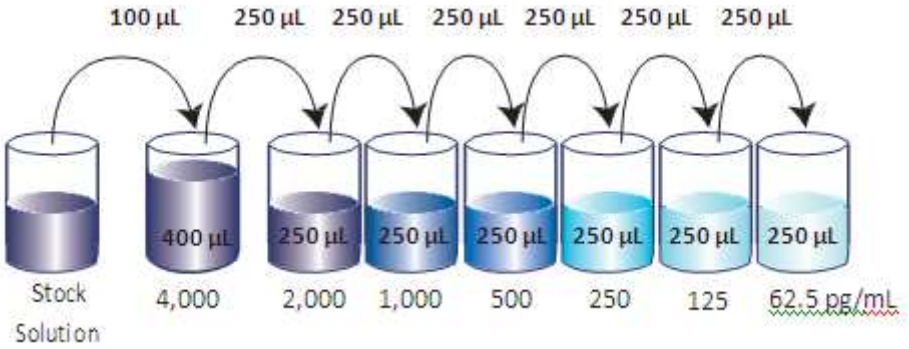


Figure 1: Schematic of serial dilutions and concentrations (pg/mL) for preparation of standards

5. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.

6. For **measuring cell culture supernatants**, add 50 uL of Assay Buffer A to each well that will contain either the standards or samples. Then add 50 uL of the standards or samples to each appropriate well.

7. For **measuring serum or plasma samples**, add 50 µL of Matrix C to each well that will contain the standard dilutions. Then add 50uL of the standards to the appropriate wells.

8. Add 50 µL of Assay Buffer to each well that will contain the serum or plasma samples. Then add 50 uL of the samples to the appropriate wells.

9. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.

10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

11. Add 100 µL of Mouse IL-10 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

12. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

13. Add 100 µL of Avidin-HRP A solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

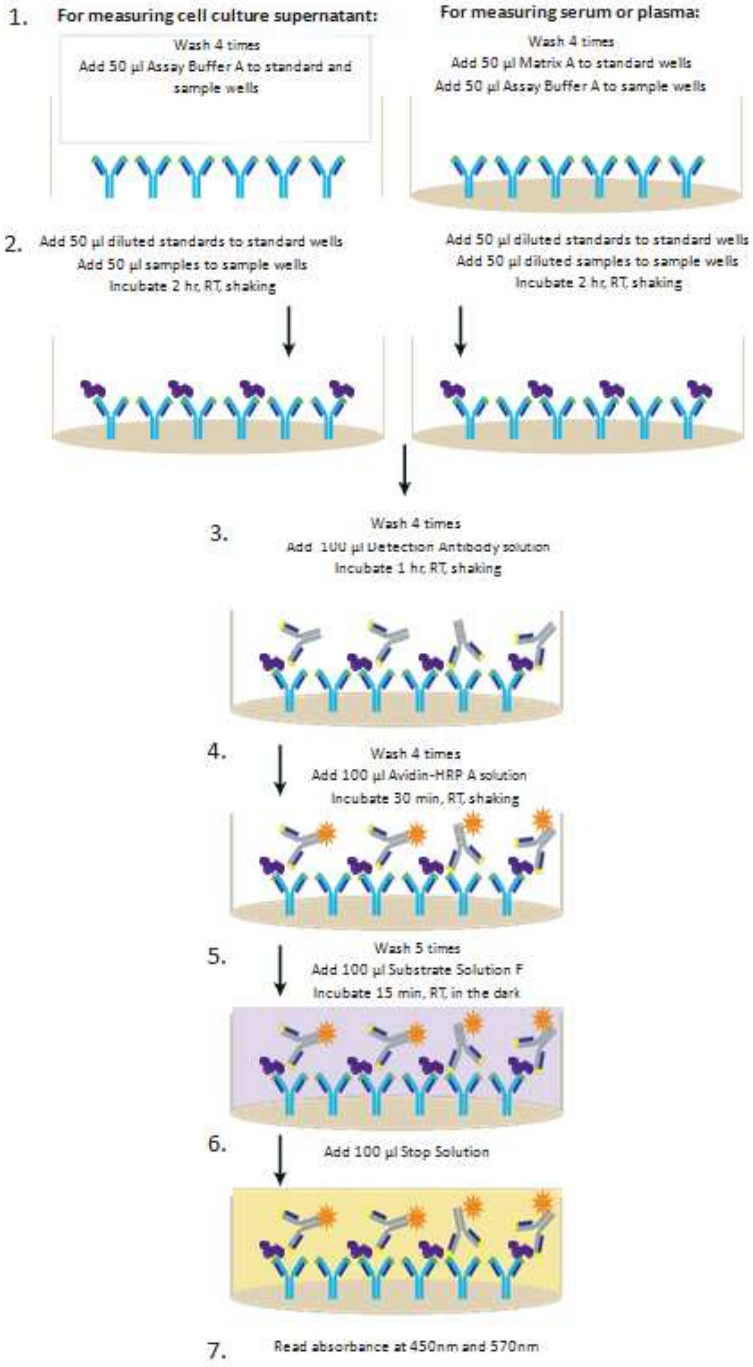
14. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

15. Add 100 µL of Substrate Solution F to each well and incubate for 15 minutes in the dark. Wells containing mouse IL-10 should turn blue in color with intensity proportional to its concentration. It is not necessary to seal the plate during this step.

16. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

17. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary



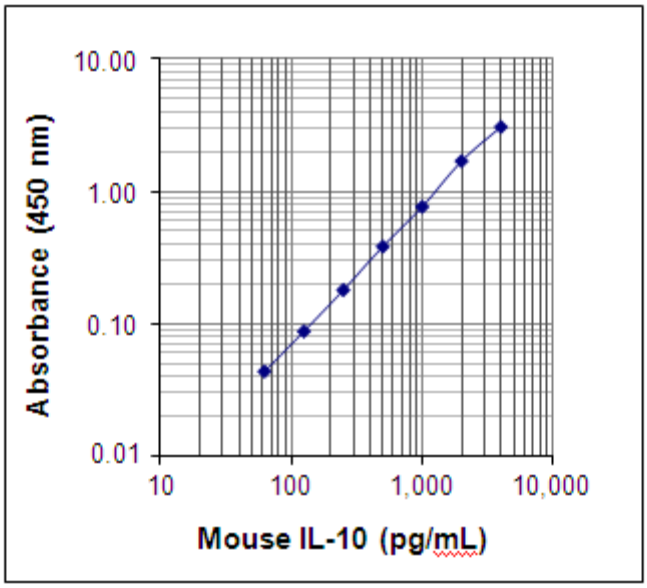
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the cytokine concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity

No cross-reactivity was observed when this kit was used to analyze 14 mouse recombinant cytokines/chemokines at up to 50 ng/mL.

Sensitivity

The minimum detectable concentration of IL-10 is 23.8 pg/mL.

Recovery

Recombinant IL-10 (2000 and 1000 pg/mL) was spiked into 4 mouse serum samples and then analyzed with Mouse IL-10 ELISA kit. On average, 97.9 % of the cytokine was recovered from serum samples.

Linearity

Four murine serum samples, each from a different strain, were spiked with high concentrations of IL-10 then diluted with Matrix C to produce samples with concentrations within the dynamic range and then assayed. On average, 102 % of the expected cytokine concentration was detected from the serum samples.

Intra-Assay Statistics

Sixteen replicates of each of two samples containing different IL-10 concentrations were tested in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	1006	235
Standard Deviation	48	10
% CV	4.8	4.3

Inter-Assay Statistics

Two samples containing different concentrations of IL-10 were tested in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	1038	241
Standard Deviation	61	17
% CV	5.9	7.1

Biological Samples

Purified mouse naïve $CD4^+$ T cells (1×10^6 cells/mL) were cultured with 10 µg/mL plate-coated anti-CD3 antibody and 1 µg/mL soluble anti-CD28 antibody at 37℃ for 3 days. Cell culture supernatants were collected and assayed for levels of natural mouse IL-10. The resulting IL-10 concentration averaged 4,512 pg/mL in anti-CD3/anti-CD28-stimulated samples and there was no detectable IL-10 in unstimulated samples.

Trouble Shooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Trouble Shooting Guide:

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

Microtiter Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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