BioLegend®

Enabling Legendary Discovery™

Mouse IL-10

ELISA MAX™ Standard Set

Cat. No. 431411

BioLegend's ELISA MAX™ Standard Set contains the capture and detection antibodies, recombinant protein standard, and Avidin-HRP required for the accurate quantification of natural and recombinant mouse IL-10. BioLegend's ELISA MAX™ Standard Sets are cost-effective and designed for experienced ELISA users. Optimization of reagent concentrations and assay conditions may be required.

It is highly recommended that the instruction sheet be read in its entirety before using this product. Use the recommended assay protocol, microwell plates, buffers, diluent, and substrate solution to obtain desired assay results. Do not use this set beyond the expiration date.

Materials Provided

1. Mouse IL-10 ELISA Capture Antibody (200X)
2. Mouse IL-10 ELISA Detection Antibody (200X)
3. Mouse IL-10 Standard
4. Avidin-HRP (1000X)
5. Lot-Specific Certificate of Analysis/ELISA MAX™ Standard Set Protocol

Introduction

Mouse IL-10 was originally described as Cytokine Synthesis Inhibitory Factor (CSIF) due to its ability to inhibit cytokine production by T_H1 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. The functions of IL-10 include inhibition of macrophage-mediated cytokine synthesis and suppression of the delayed type hypersensitivity response.

Principle of the Test

BioLegend's ELISA MAX™ Standard Set is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). A mouse IL-10 specific rat monoclonal antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and IL-10 binds to the immobilized capture antibody. Next, a biotinylated rat monoclonal anti-mouse IL-10 detection antibody is added, producing an antibody-antigen-antibody "sandwich". Avidin-horseradish peroxidase is subsequently added, followed by TMB Substrate Solution, producing a blue color in proportion to the concentration of IL-10 present in the sample. Finally, the Stop Solution changes the reaction color from blue to yellow, and the microwell absorbance is read at 450 nm with a microplate reader.

For research purposes only. Not for use in diagnostic or therapeutic procedures.

Materials to be Provided by the End-User

- Microwell plates: BioLegend Cat. No. 423501 is recommended
- Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20
- Assay Diluent: 10% Fetal Bovine Serum or 1% BSA in PBS, pH 7.4, 0.2 µM filtered. (BioLegend Cat. No. 421203 is recommended)
- Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, e.g. 2N H_2SO_4
- Plate Sealers: BioLegend Cat. No. 423601 is recommended
- PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 µm filtered
- Deionized (DI) water
- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 µL to 1 mL
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer

Storage Information

- Store kit components between 2°C and 8°C.
- After reconstitution of the lyophilized standard with Assay Diluent, aliquot into polypropylene vials and store at -70°C. Do not repeatedly freeze/thaw the recombinant protein standard as loss of activity may occur.
- Prior to use, bring all components to room temperature (18°C-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details (www.biolegend.com/msds).
2. TMB substrate solution is harmful if ingested. Additionally, avoid skin, eye or clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. 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 < -20°C.
Avoid repeated freeze/thaw cycles. Serum specimens should be clear and non-hemolyzed.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 X *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze/thaw cycles. Plasma specimens should be clear and non-hemolyzed.

Reagent and Sample Preparation

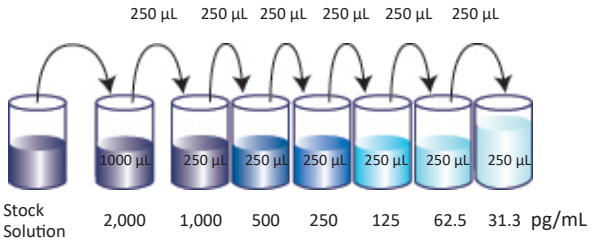
Do not mix reagents from different sets or lots. Avidin-HRP, Mouse IL-10 Standard, and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

Preparation of 1X Reagent for 1 Plate

Material	Dilute with
60 µL of Capture Antibody (200X)	12 mL of PBS, pH 7.4
60 µL of Detection Antibody (200X)	12 mL of Assay Diluent
12 µL of Avidin-HRP (1,000X)	12 mL of Assay Diluent

Standard: Lyophilized vials are under vacuum pressure. **Refer to Lot-Specific Certificate of Analysis** for Standard Reconstitution. Allow the reconstituted standard to sit fo 15 minutes at room temperature, then mix gently prior to making dilutions.

Prior to use, refer tp **Lot-Specific Certificate of Analysis** for preparation of 1,000 µL of the top standard at a concentration of 2,000 pg/mL from stock solution in Assay Diluent. Perform six two-fold serial dilutions of the 2,000 pg/mL top standard with Assay Diluent in separate tubes. After diluting, the mouse IL-10 standard concentrations are 2,000 pg/mL, 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, and 31.3 pg/mL, respectively. Assay Diluent serves as the zero standard (0 pg/mL).



Samples: For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. If dilutions are necessary, samples should be diluted in the corresponding cell culture medium.

For other sample types, such as serum and plasma, optimization of reagent concentrations and assay conditions may be required.

Assay Procedure

Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.

1. One day prior to running the ELISA, dilute Capture Antibody in **PBS, pH 7.4**. **Do not use pH 9.5 Carbonate Buffer as the coating buffer for this assay.** Add 100 µL of this Capture Antibody solution to all wells of a 96-well plate. Seal plate and incubate overnight between 2°C and 8°C.
2. Bring all reagents to room temperature (RT) 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. Wash plate 4 times with at least 300 µL Wash Buffer per well and blot residual buffer by firmly tapping plate upside down on absorbent paper. **All subsequent washes should be performed similarly.**
4. To block non-specific binding and reduce background, add 200 µL Assay Diluent per well.
5. Seal plate and incubate at RT for 1 hour with shaking on a plate shaker(e.g. 500 rpm with a 0.3 cm circular orbit). All subsequent incubation with shaking should be performed similarly.

6. While plate is being blocked, prepare standard dilutions and appropriate sample dilutions (if necessary).
7. Wash plate 4 times with Wash Buffer.
8. Add 100 µL/well of standard dilutions and samples to the appropriate wells. If needed, samples can be further diluted with Assay Diluent before adding 100 µL/well diluted samples.
9. Seal plate and incubate at RT for 2 hours with shaking.
10. Wash plate 4 times with Wash Buffer.
11. Add 100 µL of diluted Detection Antibody solution to each well, seal plate and incubate at RT for 1 hour with shaking.
12. Wash plate 4 times with Wash Buffer.
13. Add 100 µL of diluted Avidin-HRP solution to each well, seal plate and incubate at RT for 30 minutes with shaking.
14. Wash plate 5 times with Wash Buffer. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
15. Add 100 µL of TMB Substrate Solution and incubate **in the dark for 20-30 minutes or until the desired color develops*.** Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
16. Stop reaction by adding 100 µL of Stop Solution to each well. Positive wells should turn from blue to yellow.
17. Read absorbance at 450 nm within 15 minutes. If the reader can read at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

***Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.**

Assay Procedure Summary

Day 1

Add 100 µL diluted Capture Antibody solution to each well, incubate overnight between 2°C and 8°C.

Day 2

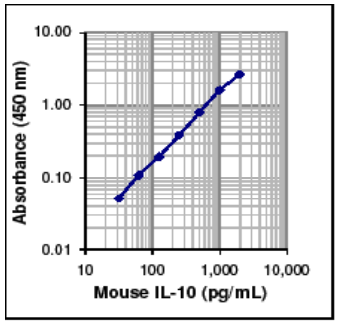
1. Wash plate 4 times
2. Add 200 µL Assay Diluent to block, incubate at room temperature for 1 hour with shaking
3. Wash plate 4 times
4. Add diluted standards and samples to the appropriate wells, incubate at room temperature for 2 hours with shaking
5. Wash plate 4 times
6. Add 100 µL diluted Detection Antibody solution to each well, incubate at room temperature for 1 hour with shaking
7. Wash plate 4 times
8. Add 100 µL diluted Avidin-HRP solution to each well, incubate at room temperature for 30 minutes with shaking
9. Wash plate 5 times, soaking for 30 seconds to 1 minute per wash
10. Add 100 µL of TMB Substrate Solution to each well, incubate in the dark for 20-30 minutes or until the desired color develops
11. Add 100 µL Stop Solution to each well
12. Read absorbance at 450 nm and 570 nm

Calculation of Results

Plot the standard curve on log-log axis 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 in the samples, find the absorbance value of the unknown 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 corresponding cytokine concentration. If the samples were diluted, multiply by the appropriate dilution factor. The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If a test sample's absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at a higher or lower dilution as appropriate.

Typical Data

Standard Curve: This standard curve was generated at BioLegend 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 multiple human, mouse and rat recombinant proteins.

For more information about BioLegend ELISA MAX™ Sets and LEGEND MAX™ ELISA Kits with precoated plates, visit **www.biolegend.com.**

Troubleshooting

High Background:

- Background wells were contaminated.
- Matrix used had endogenous analyte.
- Plate was insufficiently washed.
- TMB Substrate Solution was contaminated.

No signal:

- Incorrect or no antibodies were added.
- Avidin-HRP was not added.
- Substrate solution was not added.
- Wash buffer contained sodium azide.

Low or poor signal for the standard curve:

- Standard was incompletely reconstituted or was stored improperly.
- Reagents were added to wells with incorrect concentrations.
- Plate was incubated with inappropriate temperature, timing or agitation.

Signal too high, standard curves saturated:

- Standard was reconstituted with less volume than required.
- One or more reagent incubation steps were too long.
- Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

- Samples contain no or below detectable levels of analyte.
- Samples contain analyte concentrations greater than highest standard point.

High variations in samples and/or standards:

- Pipetting errors may have occurred.
- Plate washing was inadequate or nonuniform.
- Samples were not homogenous.
- Samples or standard wells were contaminated.

References Using This Set

1. Inoue, S., et al. 2006. Cancer Research 66:7741.
2. Lau, A.H., et al., 2006. J. Leukoc. Biol. 79:941.

BioLegend, Inc.

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For other technical resources, please visit: **www.biolegend.com/support** or email: **techserv@biolegend.com**