### Assay Procedure Summary

1. Coat plate with 100 μL diluted Capture Antibody 
   Incubate overnight, 4°C

2. Wash 4 times
   Add 200 μL 1X Assay Diluent A 
   Incubate 1 hr, RT, shaking

3. Wash 4 times
   Add 100 μL diluted standards and samples 
   Incubate 2 hrs, RT, shaking

4. Wash 4 times
   Add 100 μL diluted Detection Antibody 
   Incubate 1 hr, RT, shaking

5. Wash 4 times
   Add 100 μL Avidin-HRP 
   Incubate 30 min. RT, shaking

6. Wash 5 times 
   Add 100 μL TMB Substrate Solution 
   Incubate 30 min. RT, in the dark

7. Add 100 μL Stop Solution

8. Read absorbance at 450 nm and 570 nm

### Performance Characteristics

**Sensitivity:** The expected minimum detectable concentration of IL-10 for this set is 2 pg/mL.

**Specificity:** No cross reactivity was observed when this kit was used to analyze multiple human, mouse and rat recombinant proteins.

### 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 contains 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 improper 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.

### Human IL-10

**ELISA MAX™ Deluxe Sets**

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<th>Cat. No.</th>
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BioLegend’s ELISA MAX™ Deluxe Sets contain the components necessary for the accurate quantification of natural and recombinant human IL-10. These sets are designed for cost-effective and accurate quantification of human IL-10 in cell culture supernatant, serum, plasma or other biological fluids. They are sensitive, accurate, and robust.

**It is highly recommended that this instruction sheet be read in its entirety before using this product. Do not use this set beyond the expiration date.**

### Materials Provided

1. Human IL-10 ELISA MAX™ Capture Antibody (200X)
2. Human IL-10 ELISA MAX™ Detection Antibody (200X)
3. Human IL-10 Standard
4. Avidin-HRP (1000X)
5. Substrate Solution A
6. Substrate Solution B
7. Coating Buffer A (5X)
8. Assay Diluent A (5X)
9. NUNC Maxisorp™ 96 MicroWell Plates
10. Instruction Sheet
11. Lot-Specific Instruction/ Analysis Certificate

### Introduction

Human IL-10 was originally described as Cytokine Synthesis Inhibitory Factor (CSIF) due to its ability to inhibit cytokine production by T<sub>H</sub>1 clones. IL-10 is expressed in activated CD8<sup>+</sup> and CD4<sup>+</sup> T cells, activated monocytes, mast cells, and Ly-1 B cells. IL-10 shares over 80% sequence homology with the Epstein-Barr virus protein BCRF1. 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™ Deluxe Set is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). A human IL-10 specific 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 anti-human 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.
Materials to be Provided by the End-User

- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 μL to 1 mL
- Deionized (DI) water
- PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g Na₂HPO₄, 0.2 g KH₂PO₄, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 μm filtered
- Wash Buffer (Phosphate-Buffered Saline (PBS) + 0.05% Tween-20, pH 7.4). BioLegend Cat. No. 421601 is recommended.
- Wash bottle or automated microplate washer
- Stop Solution (2N H₂SO₄)
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Sealer
- Absorbent paper

Storage Information

- Store kit components at 4°C.
- After reconstitution of the lyophilized standard with 1X Assay Diluent A, aliquot into polypropylene vials and store at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
- Prior to use, bring all components to room temperature (18-25°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/support/#msds).
2. Substrate Solution A and Substrate Solution B are 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 Preparation

Do not mix reagents from different sets or lots. Reagents and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

1. Dilute 5X Coating Buffer to 1X with deionized water. For one plate, dilute 2.4 mL 5X Coating Buffer in 9.6 mL deionized water.
2. Dilute pre-titrated Capture Antibody 1:200 in 1X Coating Buffer. For one plate, dilute 60 μL Capture Antibody in 11.94 mL 1X Coating Buffer.
3. Dilute 5X Assay Diluent A to 1X with PBS (pH 7.4). For 50 mL, dilute 10 mL 5X Assay Diluent A in 40 mL PBS.
   NOTE: Precipitation of 5X Assay Diluent A may be observed when stored long term at 4°C. The precipitation does not alter the performance of the Buffer. If heavy precipitation is observed after the dilution to 1X Assay Diluent A, it can be filtered to clarify the solution.
4. Lyophilized vials are under vacuum pressure. Reconstitute lyophilized standard with 0.2 mL of 1X Assay Diluent A. Allow the reconstituted standard to sit for 15 minutes at room temperature, then mix gently prior to making dilutions.
5. Prior to use, prepare 1,000 μL of the top standard at a concentration of 250 pg/mL from the stock solution in 1X Assay Diluent A (refer to Lot-Specific Instruction/Analysis Certificate).
6. Dilute the pre-titrated Biotinylated Detection Antibody 1:200 in 1X Assay Diluent A. For one plate, dilute 60 μL Detection Antibody in 11.94 mL 1X Assay Diluent A.
7. Dilute Avidin-HRP 1:1000 in 1X Assay Diluent A. For one plate, dilute 12 μL Avidin-HRP in 11.99 mL 1X Assay Diluent A.
8. TMB Substrate Solution A is a mixture of equal volumes of Substrate Solution A with Substrate Solution B. Mix the two components immediately prior to use. For one plate mix 6 mL Substrate Solution A with 6 mL of Substrate Solution B in a clean container (solution should be clear and colorless).

Calculation of Results

Plot the standard curve on log-log axis graph paper with analyte concentration on the x-axis and absorbance on the y-axis. Draw a best fit line through the standard points. To determine the unknown analyte 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 analyte concentration. If the samples were diluted, multiply by the appropriate dilution factor. The data is best calculated by 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.