# Product Manual

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# Mouse Interleukin 10 (IL10) ELISA Kit

**Catalog No.:** abx050095

**Size:** 96T

**Range:** 15.6 pg/ml - 1000 pg/ml

**Sensitivity:** < 1 pg/ml

**Storage:** Store at 2-8°C for 6 months, or at -20°C for 12 months.

**Application:** For quantitative detection of IL10 in Mouse Serum, Cell Culture Supernatants, Cell Lysates and other biological fluids.

**Introduction:** Interleukin-10 (IL-10 or IL10) also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine. In humans, IL-10 is encoded by the IL10 gene, which is located on chromosome 1 and comprises 5 exons. The IL-10 protein is a homodimer, each of its subunits is 178-amino-acid long. It is primarily produced by monocytes. IL-10 is a cytokine with pleiotropic effects in immunoregulation and inflammation. It downregulates the expression of Th1 cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production.

**Principle of the Assay**

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Anti-IL10 is pre-coated onto a 96-well plate. Biotin conjugated anti-IL10 antibody is used as a detection antibody. The standards, test samples and biotin conjugated antibody are added to the wells and washed with wash buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the IL10 amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of IL10 can be calculated.

**Kit components**

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Standard: 2 tubes
3. Sample / Standard diluent buffer: 30 ml
4. Biotin conjugated antibody: 130 µl - Dilution: 1:100
5. Antibody diluent buffer: 12 ml
6. Avidin-Biotin-Peroxidase Complex (ABC): 130 µl - Dilution: 1:100
7. ABC diluent buffer: 12 ml
8. TMB substrate: 10 ml
9. Stop solution: 10 ml
10. Wash buffer (25X): 30 ml

**Material Required But Not Provided**

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

## Protocol

### A. Preparation of sample and reagents

#### 1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Allow the serum to clot in a serum separator tube (about 30 min) at room temperature. Centrifuge at approximately 1000 x g for 10 min. Analyze the serum immediately or aliquot and store frozen at -20°C or -80°C.
- **Cell culture supernate, tissue lysate or body fluids:** Remove particulates by centrifugation, analyze immediately or aliquot and store frozen at -20°C or -80°C.
- **Cell lysates:** Detach adherent cells with trypsin and collect by centrifugation and remove the supernatant. Wash the cells three times in ice-cold PBS and re-suspend cells in PBS. Lyse the cells by ultra-sonification 4 times or freeze at -20°C and thaw to room temperature 3 times. Centrifuge at 1500 × g for 10 min at 2-8°C to remove cellular debris. Collect the supernatant and assay immediately.

**Note:**

» Samples must be diluted so that the expected concentration falls within the kit's range.

» Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.

» $NaN_3$ cannot be used as test sample preservative, since it inhibits HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

**General Sample guideline:**

Estimate the concentration of the target in the sample and select the correct dilution factor to make the diluted target concentration fall near the middle of the kit's range. Generally, for high concentration (10000 pg/ml - 100000 pg/ml), dilute 1:100, for medium concentration (1000 pg/ml - 10000 pg/ml), dilute 1:10 and for low concentration (15.6 pg/ml - 1000 pg/ml), dilute 1:2. Very low concentrations (≤ 15.6 pg/ml ) do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

#### 2. Wash buffer

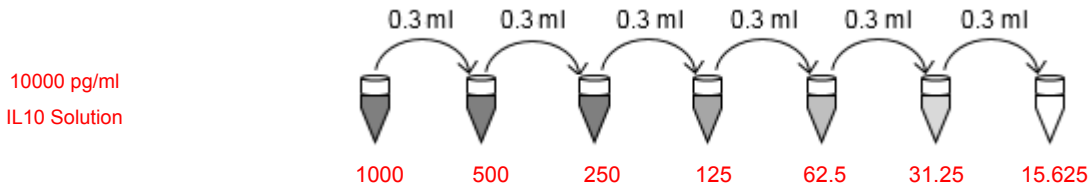
Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

#### 3. Standard

The standard solution should be prepared no more than 2 hours prior to the experiment. Note: Do not dilute the standard directly in the plate. Two tubes of standard are included in each kit. Use one tube for each experiment.

a.) 10,000 pg/ml of standard solution: Add 1 ml of Sample/Standard diluent buffer into one Standard tube to make the 10,000 pg/ml solution. Keep the tube at room temperature for 10 min, mix thoroughly and avoid foaming or bubbles. Further dilute the above solution by a factor of 10 to give the highest standard (1000 pg/ml).

b.) 1000 pg/ml → 15.625 pg/ml standard solutions: Label 6 tubes with 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml and 15.625 pg/ml. Aliquot 0.3 ml of the Sample / Standard diluent buffer into each tube. Add 0.3 ml of the above 1000 pg/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.3 ml from the 1st tube to the 2nd tube and mix thoroughly. Transfer 0.3 ml from the 2nd tube to the 3rd tube and mix thoroughly, and so on.




**Note:** Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard.

**4. Preparation of Biotin conjugated antibody working solution:** prepare no more than 2 hour before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the Biotin conjugated antibody with Antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated antibody into 99 µl of Antibody diluent buffer.

**5. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution:** prepare no more than 1 hour before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the ABC with ABC diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of ABC into 99 µl of ABC diluent buffer.

**B. Assay Procedure**
The ABC working solution and TMB substrate must be kept warm at 37 °C for 30 min before use. It is recommend to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommend to measure each standard and sample in duplicate or triplicate. Add the solution at the bottom of each well without touching the side walls.
2. Aliquot 0.1 ml of the diluted standards into the standard wells.
3. Add 0.1 ml of Sample / Standard diluent buffer into the control (zero) well.
4. Add 0.1 ml of appropriately diluted sample into test sample wells.
5. Seal the plate with a cover and incubate at 37°C for 90 min.
6. Remove the cover and discard the plate content, tap the plate on absorbent filter papers. **Do not let the wells completely dry at any time. Do not wash plate.**
7. Add 0.1 ml of Biotin conjugated antibody working solution into the wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37°C for 60 min.
9. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. Fill each well completely with Wash buffer (300µL) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
10. Add 0.1 ml of ABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
11. Remove the cover and wash the plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1-2 min. (See Step 9 for plate wash method).

12. Add 0.1 ml of TMB substrate into each well, cover the plate and incubate at 37°C in dark conditions for 15-30 min. (Note: This incubation time is for reference only, the optimal time should be determined by the end user.)
13. Add 0.1 ml of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
14. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

**For calculation:** (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The Mouse IL10 concentration of the samples can be interpolated from the standard curve.
**Note:** If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.Equilibrate the ABC working solution for at least 30 minutes to room temperature prior to use. It is recommended to plot a standard curve for each test.

**C. Precautions**

1. Before the experiment, centrifuge the tubes briefly to bring down any contents trapped in the lid.
2. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes. It is recommend to assay all standards, controls and samples in duplicate or triplicate.
3. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard solutions within 15 min of starting the experiment. Please use the diluted Standard for a single assay procedure and discard after use.
4. Do not let the plate dry out completely during the assay as this will inactivate the biological material on the plate. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
5. Ensure plates are properly sealed or covered during incubation steps.
6. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
7. Do not reuse pipette tips and tubes to avoid cross contamination.
8. Do not use expired components or components from different batches.
9. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.