Mouse Interleukin-10 (IL-10) ELISA

Catalog Number M046050

For the quantitative determination of IL-10 in mouse plasma, serum tissue extracts and cell culture supernate samples.

For research use only.
This product insert must be read in its entirety before using this product.
SUMMARY AND EXPLANATION

Interleukin-10 (IL-10) is a regulatory cytokine, and its principal role in vivo is to limit inflammatory response. IL-10 has been shown to influence both the susceptibility and course of various diseases. Interleukin-10 (IL-10) is a key cytokine produced by a multitude of immune effector cells and possesses distinct regulatory effects on immune functioning in the skin. The accelerated alveolar bone loss observed in IL-10 (-/-) mice is a late-onset condition and that lack of IL-10 may have an effect on bone homeostasis.

PRINCIPLE OF THE ASSAY

The Mouse Interleukin-10 ELISA kit is designed for detection of IL-10 in mouse plasma, serum, tissue extracts, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures IL-10 in less than 5 hours. A murine monoclonal antibody specific for human IL-10 has been pre-coated onto a 96-well microplate with removable strips. IL-10 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human IL-10, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

KIT COMPONENTS

IL-10 Microplate - The plate contains 12 x 8-well strips coated with a monoclonal antibody against IL-10. Ready to use.
IL-10 Standard - Mouse IL-10 in a buffered protein base, lyophilized.
Biotinylated IL-10 Antibody (100x) - A 100-fold concentrated biotinylated polyclonal antibody against mouse IL-10.
MIX Diluent Concentrate (10x) - A 10-fold concentrated buffered protein base.
Wash Buffer Concentrate (20x) - A 20-fold concentrated buffered surfactant, 2 bottles.
Streptavidin-Peroxidase Conjugate (SP Conjugate) - A 100-fold concentrate.
Chromogen Substrate - Stabilized peroxidase chromogen substrate tetramethylbenzidine. Ready to use.
Stop Solution - A 0.5 N hydrochloric acid to stop the chromogen substrate reaction.
Plate Sealers: 3 adhesive strips.
STORAGE

<table>
<thead>
<tr>
<th>Unopened kit</th>
<th>The SP Conjugate, IL-10 Antibody and IL-10 Standard need to be stored at -20°C. All other reagents are stored at 2 - 8°C. Do not use past the kit expiration date.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opened/Reconstituted Reagents</td>
<td>MIX Diluent</td>
</tr>
<tr>
<td></td>
<td>Wash Buffer</td>
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<tr>
<td></td>
<td>Substrate</td>
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<tr>
<td></td>
<td>Stop Solution</td>
</tr>
<tr>
<td></td>
<td>Biotinylated Antibody</td>
</tr>
<tr>
<td></td>
<td>SP Conjugate</td>
</tr>
<tr>
<td></td>
<td>Standard</td>
</tr>
<tr>
<td></td>
<td>Microplate Wells</td>
</tr>
</tbody>
</table>

SUPPLIES REQUIRED BUT NOT PROVIDED

- Pipettes or pipetting equipment with disposable polypropylene tips
- Glass measuring cylinders
- Distilled or deionized water
- Squirt bottle or automated microplate washer
- Microplate reader capable of measuring at 450 nm
- Shaker/Vortexer

PRECAUTIONS

Stop Solution consists of diluted hydrochloric acid. Wear eye, hand, face, and clothing protection when using these materials. Avoid contact with skin and eyes. In case of contact wash immediately with water. All chemicals should be considered as being potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice.

- For research use only. Not for internal or external use in humans or animals.
- This kit contains no material of human origin.
- For the handling of blood (serum), we recommend that precautions should be observed.
- Please refer to HHS Publication no. (CDC) 88-8395 or corresponding local/ national guidelines on laboratory safety procedures.
SAMPLE COLLECTION & STORAGE

**Plasma** - Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as an anticoagulant)

**Serum** - Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes. Remove serum and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

**Cell Culture Supernatants** - Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

**Tissue** - Extract tissue samples with 0.1 M Tris-buffered saline (pH7.4) containing 0.5% Triton X-100 and centrifuge at 14000 x g for 30 minutes. Collect the supernatant, measure the protein concentration and assay. Store the remaining extract at -20°C or below. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature (22 - 25 °C) before use. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use.

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature (22 - 25 °C) and mix gently until the crystals have completely dissolved. Dilute Wash Buffer concentrate 1:20 with deionized water. Store at 2 - 8 °C.

**MIX Diluent** - If crystals have formed in the concentrate, warm to room temperature (22 - 25 °C) and mix gently until the crystals have completely dissolved. Dilute MIX Diluent 1:10 with deionized water. Store for up to 30 days at 2 - 8 °C.

**Conjugate** - Dilute desired amount of Conjugate Concentrate 1:100 with MIX Diluent. Store remaining solution at -20°C.

**Biotinylated Antibody** - Dilute desired amount of Antibody 1:100 with MIX Diluent. Store remaining solution at -20 °C.

**Standards** - Reconstitute the 2 ng of Mouse IL-10 standard with 1 mL of MIX Diluent. This reconstitution produces a stock solution of 2 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (2 ng/mL) 1:2 with equal volume of MIX Diluent to produce 1, 0.5, 0.25, 0.125, 0.063 and 0.031 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20°C and used within 30 days.

![Serial Dilutions using 150 μL](image-url)
ASSAY PROTOCOL

Read the entire protocol before beginning the assay. It is recommended that all standards and samples be assayed in duplicate. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use. Note: Reagents and samples may require specific handling temperatures and need preparation prior to the assay. See the Reagent and Sample Preparation sections before proceeding.

Note: Bring all reagents and samples to room temperature (22 - 25 °C) before use.

1. Prepare all reagents and samples as described in the previous sections.
2. Remove any excess microplate strips from the plate frame and return them to the foil pouch containing the desiccant pack.
3. Pipette 50 µL of Standard or sample in duplicate into the wells using a clean pipette tip for each standard or sample. Cover with the plate sealer provided and incubate for 2 hours at room temperature (22 - 25 °C).
4. Aspirate and wash the wells 5 times with 200 µL per well of Wash Buffer. Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
5. Add 50 µL of Biotinylated Antibody into each well. Cover with the plate sealer provided and incubate for 2 hours at room temperature (22 - 25 °C).
6. Aspirate and wash the wells 5 times with 200 µL per well of Wash Buffer. Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
7. Add 50 µL of Conjugate to each well. Incubate for 30 minutes at room temperature (22 - 25 °C).
8. Aspirate and wash the wells 5 times with 200 µL per well of Wash Buffer. Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
9. Add 50 µL Substrate to each well and incubate for 15 minutes at room temperature (22 - 25 °C) or until optimal color density develops. Gently tap the side of the plate to ensure thorough mixing.
10. Stop the reaction by adding 50 µL of Stop Solution to each well.
11. Read the plate at 450 nm. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. If it is not available, read plate at 450 nm only. Note: some unstable black particles may be generated at high concentration points after stopping the reaction at 10 minutes, which may reduce the absorbance readings.
SUMMARY

Prepare reagents and samples as previously described.

↓

Pipette 50 µL Standard or sample in duplicate into the wells. Incubate 2 hrs. at RT.

↓

Aspirate and wash 5 times.

↓

Add 50 µL of Biotinylated Antibody to each well. Incubate 2 hrs. at RT.

↓

Aspirate and wash 5 times.

↓

Add 50 µL Conjugate to each well. Incubate for 30 min. at RT.

↓

Aspirate and wash 5 times.

↓

Add 50 µL of Substrate to each well. Incubate 15 min. at RT.

↓

Add 50 µL of Stop Solution to each well. Read at 450 nm.
CALCULATION OF RESULTS

Calculate the mean value of the duplicate or triplicate readings for each standard and sample. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis of the linear portion of the curve. Determine the unknown sample concentration from the Standard Curve and multiply the dilution factor.

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

PERFORMANCE CHARACTERISTICS

Sensitivity

Sensitivity is defined as the minimal detectable dose determined by adding two standard deviations of the mean optical density value for replicates of the zero standard and calculating the corresponding concentration. The sensitivity of the Mouse Interleukin-10 (IL-10) ELISA is typically ~ 0.03 ng/mL.

Reproducibility

Intra-assay Precision (Precision within an assay) - The intra-assay precision was measured by assaying control samples multiple times on one plate. The intra-assay precision coefficient of variation (CV) is 4.9%.

Inter-assay Precision (Precision between assays) - The inter-assay precision was assessed by repeated measurements of control samples in successive assays with multiple users. The inter-assay precision coefficient of variation (CV) is 7.1%.
### Linearity

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>Plasma (%)</th>
<th>Serum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Dilution</td>
<td>95%</td>
<td>97%</td>
</tr>
<tr>
<td>1:2</td>
<td>100%</td>
<td>101%</td>
</tr>
<tr>
<td>1:4</td>
<td>98%</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Recovery

<table>
<thead>
<tr>
<th>Standard Added Value</th>
<th>0.01 - 1.0 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (%)</td>
<td>82 - 117%</td>
</tr>
<tr>
<td>Average Recovery (%)</td>
<td>99.5%</td>
</tr>
</tbody>
</table>

### Cross-Reactivity

<table>
<thead>
<tr>
<th>Species</th>
<th>Cross-reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>0%</td>
</tr>
<tr>
<td>Canine</td>
<td>0%</td>
</tr>
<tr>
<td>Human</td>
<td>0%</td>
</tr>
<tr>
<td>Monkey</td>
<td>0%</td>
</tr>
<tr>
<td>Mouse</td>
<td>100%</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0%</td>
</tr>
<tr>
<td>Rat</td>
<td>0%</td>
</tr>
<tr>
<td>Swine</td>
<td>0%</td>
</tr>
</tbody>
</table>

### Reference Value

Normal mouse IL-10 plasma levels is <20ng/mL.
REFERENCES


RELATED PRODUCTS

- Human IL-10 ELISA Kit (Plasma, Serum, Cell Culture Supernatants, and Tissue samples).
- Rat IL-10 ELISA Kit (Cell Culture Supernatant samples).
NOTES
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