

Rat Anti-Mouse Interleukin-6 (IL-6) ELISA Set

Cat. No.	Form	Quantity
14300-01	ELISA Set	1

For Research Use Only

Intended Use

This set is specifically designed for quantitative determination of mouse interleukin 6 (IL-6) concentrations in cell culture supernatant and serum.

Background

Interleukin 6 (IL-6) is a 22-28kD α -helical protein that is secreted by a variety of cells including monocytes, fibroblasts, B and T cells, endothelial cells and glial cells¹. This cytokine signals through a receptor complex consisting of the IL-6 receptor subunit and the ubiquitously expressed signal transducing subunit gp130². On target cells, IL-6 binding induces gp130 recruitment and subsequent activation of the MAPK and Jak/Stat signaling pathways. IL-6 stimulates B cells into antibody secreting cells and acts as a growth factor for numerous cell types including hematopoietic precursor cells, T cells, neuronal cells and hybridoma cells³⁻⁵. IL-6 has also been linked to thymocyte development and the induction of acute phase response in hepatocytes^{6,7}.

Principle of Assay

This assay incorporates a quantitative sandwich enzyme immunoassay technique. Unlabeled monoclonal antibody specific for mouse interleukin 6 (IL-6) is first coated onto 96 well microplate(s). Standards and samples are then added to the wells and any IL-6 present is captured by the immobilized antibody. After a wash to remove unbound material, biotin-labeled anti-mouse IL-6 detection antibody is added to the wells, followed by a horseradish peroxidase-labeled anti-biotin antibody incubation. After another wash, TMB substrate solution is added that will result in a blue color proportional to the amount of bound IL-6. Color development is then quenched and intensity is measured at 450nm.

Research Applications

Enzyme-Linked-Immunosorbent-Assay (ELISA)

Characterization

To ensure lot-to-lot consistency, each batch of product is tested by ELISA to conform to the characteristics of a standard reference reagent.

Assay Kit Components

- 0.3mL IL-6 Capture Antibody: unlabeled, 200X
- 0.06mL IL-6 Detection Antibody: biotin-conjugated, 1000X
- 0.3mL Revealing Antibody: HRP-conjugated, 200X

- 2 vials Standard: lyophilized recombinant mouse IL-6, 5ng/vial
- 15mL Coating Buffer (5X)
- 300mL Assay Diluent (1X)
- 300mL Washing Buffer (20X)
- 60mL Standard/Sample Diluent (1X)
- 60mL TMB one component microwell substrate
- 60mL TMB Stop Solution
- 5 x 96-well ELISA plates

Sample Collection and Storage

Cell Culture Supernatants - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Note: Serum samples require a 200-fold dilution. A suggested 200-fold dilution is 5.0µL sample into 995µL Assay Diluent.

Reagent Preparation

All reagents should be diluted immediately prior to use. Do not add sodium azide to Assay Diluent as it inhibits the activity of horseradish-peroxidase. It is recommended that all samples, controls and standards be assayed in duplicate or triplicate.

- Dilute 5X Coating Buffer to 1X with distilled (DI) water. A suggested dilution (for one plate) consists of 2.2mL 5X Coating Buffer with 8.8mL of DI water.
- Dilute unlabeled mouse IL-6 specific Capture Antibody 200-fold with 1X Coating Buffer. A suggested dilution (for one plate) consists of 55µL Capture Antibody with 10.945mL 1X Coating Buffer.
- Dilute 20X Wash Buffer to 1X with DI water. A suggested dilution consists of 50mL 20X Wash Buffer with 950mL of DI water.
- Reconstitute 1 vial lyophilized mouse IL-6 Standard by adding 0.1mL DI water to 1 vial (50ng/mL) and gently vortex. Next, dilute IL-6 Standard 100-fold by adding 5µL 50ng/mL Standard to 495µL DI water to yield a final concentration of 500pg/mL and allow the standard to equilibrate for at least 15 minutes. Aliquot any unused standard into polypropylene vials and store at -80°C.
- Dilute biotin-labeled mouse IL-6 specific Detection Antibody 1000-fold with 1X Assay Diluent. A suggested dilution (for one plate) consists of 11µL Detection Antibody with 10.989mL 1X Assay Diluent.
- Dilute horseradish peroxidase-labeled Revealing Antibody 200-fold with 1X Assay Diluent. A suggested dilution (for one plate) consists of 55µL Revealing Antibody with 10.945mL 1X Assay Diluent.

Assay Procedure

1. Collect samples and prepare reagents as directed previously.
2. Add 100µL of diluted Capture Antibody solution to all wells of a provided 96-well plate. Incubate at 4°C overnight.
3. Wash entire plate 4 times with 1X Wash Buffer.
4. Add 300µL of 1X Assay Diluent per well to block non-specific binding and reduce background. Incubate at room temperature for 30 minutes.
5. Wash plate as in Step 3.
6. Prepare standard dilution by adding 100µL/well of appropriate dilution to the plate. To do this, perform six two-fold serial dilutions of the 500pg/mL top standard either within the plate or in separate tubes. To dilute within the plate, add 200µl of 500pg/mL mouse IL-6 Standard to row A and 100µl Standard/Sample Diluent to rows B through H. Perform serial dilution by taking 100µl standard from row A and mix with row B (see below). Then take 100µl from row B and add it to row C and continue for each subsequent row. Therefore, the final mouse IL-6 standard concentrations will be 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, 31.3pg/mL, 15.6pg/mL and 7.8pg/mL. Standard/Sample Diluent alone serves as the zero standards for row H (0pg/mL). For test samples, dilute two-fold with Standard/Sample Diluent and add 100µL/well. Incubate at room temperature for 2 hours.
7. Wash plate as in Step 3.
8. Add 100µL of diluted biotin-labeled Detection Antibody solution to each well and incubate at room temperature for 1 hour.
9. Wash plate as in Step 3.
10. Add 100µL of diluted HRP-labeled Revealing Antibody solution to each well and incubate at room temperature for 30 minutes.
11. Wash plate 6 times with 1X Wash Buffer. For this final wash, soak wells in Wash Buffer for 10 seconds for each wash. This will help minimize background.
12. Add 100µL of TMB Substrate Solution to each well. Incubate at room temperature for 10-15 minutes. The Substrate Solution should turn blue in color.
13. Add 50µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells turns green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
14. Determine the optical density of each well within 30 minutes using a microplate reader set to 450 nm.

Specificity

This assay recognizes recombinant and native mouse IL-6. No cross-reactivity or interference with other mouse proteins was observed.

Sensitivity

The minimum detectable concentration of mouse IL-6 is 0.2pg/mL.

Recovery

The recovery of mouse IL-6 spiked to levels throughout the range of the assay in serum and cell culture supernatant was evaluated by the set. At least 85% of the IL-6 was recovered from serum and 95% from cell culture supernatant.

Trouble Shooting

General technical hints:

- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- During incubation steps, shaking the plates may increase sensitivity.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue upon addition.
- Stop Solution should be added to the plate in the same order as the Substrate Solution.
- The color development in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that turn green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

Poor Signal

- Avoid sodium azide in wash buffers as it inhibits the enzymatic activity of HRP
- Verify that appropriate antibody pairs were used
- Inadequate reagent volumes added to wells
- Incorrect incubation times and/or temperature

Poor Standard Curve or Precision

- Improper standard handling and/or dilution
- Inadequate mixing of reagents
- Inadequate aspiration and/or washing of wells

References

1. Bauer, J. et al. (1988). *Blood*. **72**:1134-40.
2. Heinrich, P.C. et al. (2003). *Biochem. J.* **374**:1-20.
3. Chiu, C.P. and Lee, F. (1989). *J. Immun.* **142**:1909-15.
4. Uyttenhove, C. et al. (1988). *J. Exp. Med.* **167**:1417-27.
5. Ruef, C. et al. (1990). *Kidney Int.* **38**:249-57.
6. Iho, S. et al. (1992). *Cell. Immun.* **144**:1-10.
7. Heinrich, P.C. et al. (1990). *Biochem. J.* **265**:621-36.