Product Manual

abbexa®

Hamster Interleukin 6 (IL6) ELISA Kit

Catalog No.: abx257602

Size: 96 tests

Range: 7.8 pg/ml - 500 pg/ml

Sensitivity: < 1.95 pg/ml

Storage: Store unopened at 2-8 °C for up to 1 month. For long term storage, store the standard, HRP-Avidin and Biotin conjugated antibody at -20°C.

Application: For quantitative detection of IL6 in Hamster Serum, Plasma, Tissue Homogenates and Cell Culture Supernatants.

Introduction: Interleukin 6 (IL6) is a pro- and anti-inflammatory molecule, depending on the manner in which it was synthesized. IL6 from macrophages is used as a cytokine that contributes to the production of other acute-phase molecules that elicit a febrile response, particularly prostaglandin E2. IL6 from muscle, on the other hand, functions as an anti-inflammatory myokine and is produced after exercise. The difference between these is that during inflammation the IL6 signaling interacts with TNF and NF-κB signaling, whereas during exercise it does not. Increased levels of IL6 are associated with autoimmune diseases and some cancers. IL6 knockout mice have increased inflammatory bone destruction and poor healing.

Principle of the Assay

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. An antibody specific to IL6 is pre-coated onto a 96-well plate. The standards and samples are added to the wells and incubated. Biotin conjugated anti-IL6 antibody is used as detection antibody. Next, Avidin conjugated to HRP is added to each microplate well and incubated. Unbound conjugates are washed away with wash buffer. After TMB substrate solution is added only wells that contain IL6, biotin-conjugated antibody and enzyme-conjugated Avidin will produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the IL6 amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of IL6 can be calculated.

Kit components

1. One pre-coated 96 well plate (12 × 8 well strips)
2. Standard: 2 tubes
3. Sample diluent buffer: 50 ml
4. Biotin conjugated antibody (100X): 120 µl
5. Antibody diluent buffer: 15 ml
6. HRP-Avidin (100X): 120 µl
7. HRP-Avidin diluent buffer: 15 ml
8. TMB substrate: 10 ml
9. Stop solution: 10 ml
10. Wash buffer (25X): 20 ml
11. Plate sealer: 4

Materials Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm, correction wavelength: 540 or 570 nm)
3. High-precision pipette and sterile disposable pipette tips
4. Squirt bottle or automated plate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare samples
7. Distilled water
8. Absorbent filter papers
9. 100 ml and 1 L graduated cylinders

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Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, analyze immediately or store at 4°C for up to 5 days. Otherwise, store at -20°C for up to one month or -80°C for up to two months to avoid loss of bioactivity. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4 °C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20 °C or -80 °C.

- **Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples

- **Tissue Homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20 °C.

- **Cell culture supernatants:** Remove particulates by centrifugation, analyze immediately or aliquot and store frozen at -20 °C or -80 °C.

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.

2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 20 ml of concentrated wash buffer into 480 ml of distilled water). If crystals have formed, bring to room temperature and mix gently until the crystals have completely dissolved.

3. HRP-Avidin

Centrifuge the vial before opening. Dilute the 100X HRP-Avidin reagent 100-fold (1/100) with HRP-Avidin diluent buffer to prepare the 1X HRP-avidin working solution (i.e. add 10 μl of concentrated HRP-Avidin into 990 μl of HRP-Avidin diluent buffer).

4. Biotin conjugated antibody

Centrifuge the vial before opening. Dilute the 100X Biotin conjugated antibody 100-fold (1/100) with Antibody diluent buffer to prepare the 1X Biotin conjugated antibody working solution (i.e. add 10 μl of concentrated Biotin conjugated antibody into 990 μl of Antibody diluent buffer). If the solution appears cloudy, bring to room temperature and mix gently until the solution appears uniform.

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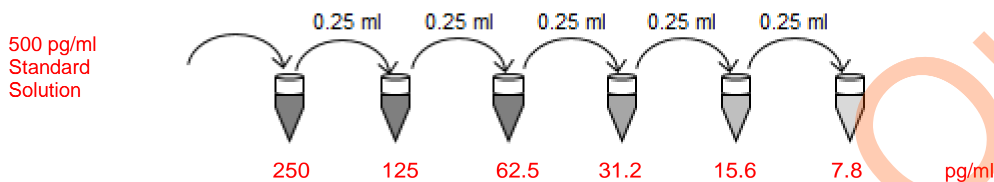
5. Standard

Centrifuge the standard vial at 6000-10,000 RPM for 30 seconds before opening. Reconstitute the standard in 1.0 ml of Sample diluent buffer. Mix gently and ensure it is fully dissolved. Allow the reconstituted standard to sit for 15 minutes with gentle agitation prior to carrying out the serial dilutions, avoiding foaming or bubbles. This solution serves as the highest standard (500 pg/ml). Label 6 tubes with 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml, 15.6 pg/ml, and 7.8 pg/ml. Aliquot 0.25 ml of Sample diluent buffer into each tube. Add 0.25 ml of 500 pg/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.25 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on. The Sample diluent buffer serves as the zero standard (0 ng/ml). The standard solutions are best used within 2 hours of preparation.



B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 100 µl of the prepared standards solutions into the standard wells.
3. Add 100 µl of Sample diluent buffer into the control (zero) well.
4. Add 100 µl of appropriately diluted sample into test sample wells.
5. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 37 °C for 2 hours.
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 the plate.**
7. Add 100 µl of working Biotin conjugated antibody solution into each well. Add the solution at the bottom of each well without touching the side wall.
8. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate at 37 °C for 1 hour.
9. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash buffer. Fill each well completely with Wash buffer (200 µl) using a multi-channel pipette or automatic washer (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 100 µl of HRP-Avidin working solution into each well, cover the plate with a new sealer and incubate at 37 °C for 1 hour.
11. Remove the cover and wash the plate 5 times with Wash buffer (see step 9). Allow the wash buffer to remain in the wells for 1-2 min for each wash. Discard the washing buffer and blot the plate onto absorbent filter papers or other absorbent material.
12. Add 90 µl of TMB substrate into each well. Cover the plate and incubate at 25°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the standard wells the reaction can be terminated.
13. Add 50 µl of Stop solution into each well to stop the enzyme reaction. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely
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.

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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 can be used for data analysis. The Hamster IL6 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. It is recommended to plot a standard curve for each test.

C. Precautions

1. Equilibrate all reagents to room temperature (18-25°C) prior to use and centrifuge the tubes briefly in case any contents are 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.
3. The Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. Avoid foaming or bubbles when mixing or reconstituting components.
5. It is recommended to assay all standards, controls and samples in duplicate or triplicate.
6. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
7. Ensure plates are properly sealed or covered during incubation steps.
8. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
9. To avoid cross contamination do not reuse pipette tips and tubes.
10. 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.
11. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling these solutions. 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.

D. Precautions

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of CALPRO were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of CALPRO were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100
Intra-Assay: CV < 8%
Inter-Assay: CV < 10%

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