The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

**Recombinant human:**
- IL-1α
- IL-1β
- IL-1 RI
- IL-1 RII

**Other recombinants:**
- mouse IL-1α
- porcine IL-1α
- rat IL-1α

**SPECIFICITY**

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.
**OTHER MATERIALS & SOLUTIONS REQUIRED**

DuoSet Ancillary Reagent Kit 2 (5 plates):
(R&D Systems, Catalog # DY008) containing 96 well microplates, plate sealers, substrate solution, stop solution, plate coating buffer (PBS), wash buffer, and Reagent Diluent Concentrate 2.

The components listed above may be purchased separately:
96 well microplates: (R&D Systems, Catalog # DY990).
Plate Sealers: (R&D Systems, Catalog # DY992).
PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.2-7.4, 0.2 μm filtered (R&D Systems, Catalog # DY006).
Wash Buffer: 0.05% Tween® 20 in PBS, pH 7.2-7.4 (R&D Systems, Catalog # WA126).
Reagent Diluent: 1% BSA in PBS, pH 7.2-7.4, 0.2 μm filtered (R&D Systems, Catalog # DY995).
Quality of BSA is critical (see Technical Hints).
Substrate Solution: 1:1 mixture of Color Reagent A (H₂O₂) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999).
Stop Solution: 2 N H₂SO₄ (R&D Systems, Catalog # DY994).

**REAGENT PREPARATION**

Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Streptavidin-HRP: Each vial contains 2.0 mL of streptavidin conjugated to horseradish-peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent.

Mouse Anti-Human IL-1α Capture Antibody: Refer to the lot-specific Certificate of Analysis (C of A) for amount supplied. Reconstitute each vial with 0.5 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the C of A.

Biotinylated Goat Anti-Human IL-1α Detection Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent to the working concentration indicated on the C of A.

Recombinant Human IL-1α Standard: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of Reagent Diluent. A seven point standard curve using 2-fold serial dilutions in Reagent Diluent is recommended. Prepare 1000 μL of high standard per plate assayed at the concentration indicated on the C of A.

**CALIBRATION**

This DuoSet is calibrated against a highly purified E. coli-expressed recombinant human IL-1α produced at R&D Systems.

**MATERIALS PROVIDED & STORAGE CONDITIONS**

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

**GENERAL ELISA PROTOCOL**

**Plate Preparation**

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 μL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.

2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

3. Block plates by adding 300 μL of Reagent Diluent to each well. Incubate at room temperature for a minimum of 1 hour.

4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

**Assay Procedure**

1. Add 50 μL of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.

2. Repeat the aspiration/wash as in step 2 of Plate Preparation.

3. Add 100 μL of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.

4. Repeat the aspiration/wash as in step 2 of Plate Preparation.

5. Add 100 μL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.

6. Repeat the aspiration/wash as in step 2.

7. Add 100 μL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.

8. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

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