Human IL-1ra CytoSet™
10 Plate Format
Lot-specific Technical Data Sheet

Catalog # : CHC1183

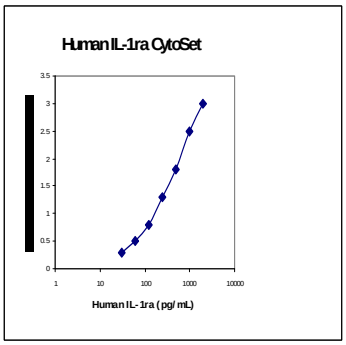
Lot # : 074001
Expiration : 31.12.2008

1. **Coating Antibody:** **Anti-Human IL-1ra (125 µg/0.125 mL)**
   Part Number: 58.118.09
   Lot Number: **6D1/1**
   Form: Liquid, 2 vials, contains 0.1% sodium azide
   Storage: Store at 2-8ºC until expiration date.
   Recommended Dilution: Dilute to 2 µg/mL with Coating Buffer A (Cat. # CB07100, or see Recommended Buffers). For example, to make 10 mL (enough to coat 1 plate), add 20 µL coating antibody to 9.980mL Coating Buffer B.

2. **Detection Antibody:** **Anti- Human IL-1ra Biotin (25 µg/0.125mL)**
   Part Number: 58.118.03
   Lot Number: **7D1/1**
   Form: Liquid, 1 vial, contains 0.1% sodium azide
   Storage: Store at 2-8ºC until expiration date.
   Recommended Dilution: Dilute to 0.16 µg/mL with Assay Buffer (Cat. # DS98200, or see Recommended Buffers). For example, to make enough for 1 plate, add 4.4 µL detection antibody to 4,995.6 µL Assay Buffer.

3. **Standard:** **Recombinant Human IL-1ra**
   Part Number: 58.118.10 (additional vials of standard may be purchased using this part number)
   Lot Number: **6B4/1**
   Form: Lyophilized, 3 vials
   Storage: Store at 2-8ºC.
   Reconstitution: Reconstitute with Assay Buffer (Cat. # DS98200 or see Recommended Buffers) to yield a stock of 10,000 pg/mL. After 10 minutes of rehydratation, use the standard stock immediately or aliquot in polypropylene tubes and freeze at –80ºC. *Do not store at room temperature or at 4ºC and do not subject to more than one freeze-thaw cycle.*
   Standard Curve: Dilute standard stock to 2000 pg/mL (120 µL stock plus 480 µL Assay Buffer) with Assay Buffer (Cat. # DS98200 or see Recommended Buffers). Add 300 µL Assay Buffer to 5 tubes and label as 1000, 500, 250, 125, 62.5 and 31.25 pg/mL. Make serial dilutions starting with 1000 pg/mL by transferring 300 µL of each standard to next tube and vortexing each tube. Assay Buffer should be used as the zero standard.

4. **Streptavidin-HRP:** **0.250 mL**
   Part Number: 41.000.03
   Lot Number: **7C4/1**
   Form: Liquid, 1vial, contains 0.05% thymol
   Storage: Store at 2-8ºC until expiration date.
   Recommended Dilution: Dilute to 1/2500. For example, to make enough for 1 plate, add 4 µL of streptavidin-HRP to 9.996 mL of Assay Buffer (Cat. # DS98200 or see Recommended Buffers).



Representative standard curve was generated by following the recommended assay procedure, which includes the use of the **Invitrogen CytoSet™ Buffer Set (Cat. # CNB0011)**

This product is for research use only. Not for use in diagnostic procedures.

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Intended Use and Materials Provided

The CytoSet™ for Human IL-1ra contains components required to construct an enzyme-linked immunoassay for the specific and quantitative measurement of IL-1ra. Sufficient quantities of all reagents are provided to yield 40 plates of 96 wells if the recommended assay procedure and recommended storage and handling of materials are followed as specified on this insert. The materials provided are **FOR RESEARCH USE ONLY**.

Recommended Buffers and Solutions

The Invitrogen CytoSet™ Buffer Set (Cat. # CNB0011) containing Coating Buffers A and B, Assay Buffer, Substrate Solution (TMB), Stop Solution, and Wash Buffer is recommended.

1. **Coating Buffer A:** ***Coating Buffer A (Cat. # CB07100) from Invitrogen is recommended.*** *Alternate buffer choice listed below.*
   8.0 g NaCl, 1.13 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, 0.1% ProClin™; q.s. to 1.0 L with distilled H_2O, pH to 7.4.
2. **Coating Buffer B:** ***Coating Buffer B (Cat. # CB01100) from Invitrogen is recommended.*** *Alternate buffer choice listed below.*
   4.3 g $NaHCO_3$, 5.3 g Na_2CO_3, 0.1% ProClin™; q.s. to 1.0 L with distilled H_2O, pH to 9.4.
3. **Assay Buffer:** ***Assay Buffer (Cat. # DS98200) from Invitrogen is recommended.*** *Alternate buffer choice listed below.*
   8.0 g NaCl, 1.13 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, 5.0 g bovine serum albumin (fraction V), 1 mL Tween 20 and 0.5% ProClin™ as a preservative; q.s. to 1.0 L with distilled H_2O, pH to 7.4.
4. **Wash Buffer:** ***Wash Buffer 25x (Cat. # WB01) from Invitrogen is recommended.*** *Alternate buffer choice listed below.*
   0.2 g KH_2PO_4, 1.9 g, $K_2HPO_4 .3H_2O$, 0.4 g EDTA, 0.5 mL Tween 20; q.s. to 1.0 L with distilled H_2O, pH to 7.4.
5. **Substrate Solution:** ***TMB (Cat. # SB01) from Invitrogen is recommended.*** *Alternate solution choice listed below.*
   Tetramethylbenzidine (TMB) and Hydrogen Peroxide.
6. **Stop Solution:** ***Stop Solution (Cat.# SS01100) from Invitrogen is recommended.*** *Alternate solution choice listed below.*
   1.8 N H_2SO_4.

Assay Optimization

CytoSets™ from Invitrogen are designed to be very flexible for your experiments. Consequently, the assay procedure contains only recommendations. The assay procedure has been optimized for use with tissue culture samples. However, serum and plasma samples may be used but may require that certain assay parameters be modified. Investigators are advised to determine optimal buffer formulations, concentrations and incubation times for individual applications.

Recommended Assay Procedure

1. Prepare coating solution by diluting the coating antibody. See "coating antibody" section for the recommended coating antibody dilution.
2. Coat plates with 100 µL per well of the coating solution. Cover plates and incubate overnight (12-18 hr.) at 4ºC.
3. Aspirate wells and wash 1 time with > 400 µL of Wash Buffer per well. Following wash, invert and tap on absorbent paper to remove excess liquid.
4. Block plate with 300 µL per well of Assay Buffer for 1 hour at room temperature.
5. Aspirate, invert, and tap on absorbent paper to remove excess liquid.
6. Prepare standards and sample dilutions in Assay Buffer (or in a diluent that most closely matches the matrix of your sample). For recommended dilutions and storage of the standard, see "standard" section.
7. Pipette 100 µL of standards (in duplicate) and samples into designated wells.
8. Immediately following step 7, add 50 µL of the working detection antibody into each well. For recommended dilutions, see "detection antibody" section. ***Incubate for 2 hours at room temperature with continual shaking (700 rpm).***
9. Aspirate and wash 5 times using the method in step 3.
10. Add 100 µL of the working streptavidin-HRP solution into each well. For recommended dilutions, see "streptavidin-HRP conjugate" section. ***Incubate for 30 minutes at room temperature with continual shaking (700 rpm).***
11. Aspirate and wash 5 times using the method in step 3.
12. Add 100 µL of the TMB substrate to each well. Incubate plate for 30 minutes at room temperature with continual shaking (700 rpm).
13. Add 100 µL of Stop Solution to each well.
14. Measure absorbance at 450 nm (reference absorbance: 650 nm) within 30 minutes of adding Stop Solution. Calculate results using a log-log or 4-parameter curve fit.

Additional Materials Required

- 96 well NUNC MaxiSorp microplates; NUNC Cat. # 434797.
- Pipettes, shaker and timer.
- Microplate reader with a detector that can measure absorbance at 450 nm.
- 1 L graduated cylinder; plate washer or wash bottle.
- Polypropylene tubes for standards and sample dilutions, if needed.