



## **IL-1 $\beta$ (human), ELISA kit**

Catalog #: ADI-900-130A

96 Well Kit



# Product Manual

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Please read entire booklet before proceeding with the assay.



Carefully note the handling and storage conditions of each kit component.



Please contact Enzo Life Sciences Technical Support if necessary.

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## DESCRIPTION

The IL-1 $\beta$  (human), ELISA kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human IL-1 $\beta$  in human serum, buffered solution, or cell culture medium. The assay recognizes both natural and recombinant human IL-1 $\beta$ .

## SAFETY WARNINGS & PRECAUTIONS

**FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**



Handle  
with care



Avoid  
freeze /  
thaw cycles

- All chemicals should be considered as 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. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Do not mix or substitute reagents with those from other lots or sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membrane with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or specimens that may invalidate the test, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.

- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials, as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5° C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

## **MATERIALS SUPPLIED**

1. 2 vials Human IL-1 $\beta$  Standard, lyophilized; contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume
2. 25 mL Standard Diluent Buffer
3. 1 plate Human IL-1 $\beta$  Antibody-Coated Wells, 96-well strip-well plate
4. 11 mL Human IL-1 $\beta$  Conjugate; contains 8 mM sodium azide
5. 0.125 mL Streptavidin-Peroxidase (HRP) (100X); contains 0.05% Proclin™ 300
6. 25 mL Streptavidin-Peroxidase (HRP) Diluent; contains 0.05% Proclin™ 300
7. 100 mL Wash Buffer Concentrate (25X)
8. 25 mL Stabilized Chromogen, Tetramethylbenzidine (TMB)
9. 25 mL Stop Solution
10. 3 Plate Covers, adhesive strips



# Product Manual

## STORAGE

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

## SPECIMEN COLLECTION

1. Collect samples in pyrogen/endotoxin-free tubes.
2. Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
3. Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

## OTHER MATERIALS NEEDED

1. Distilled or deionized water
2. Microtiter plate reader with software capable of measurement at or near 450 nm
3. Plate washer-automated or manual (squirt bottle, manifold dispenser, or equivalent)
4. Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

## PROCEDURAL NOTES

1. Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
2. Allow reagents to reach room temperature before use. Mix to re-dissolve any precipitated salts.

## REAGENT PREPARATION

### Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

### Pre-dilute Samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

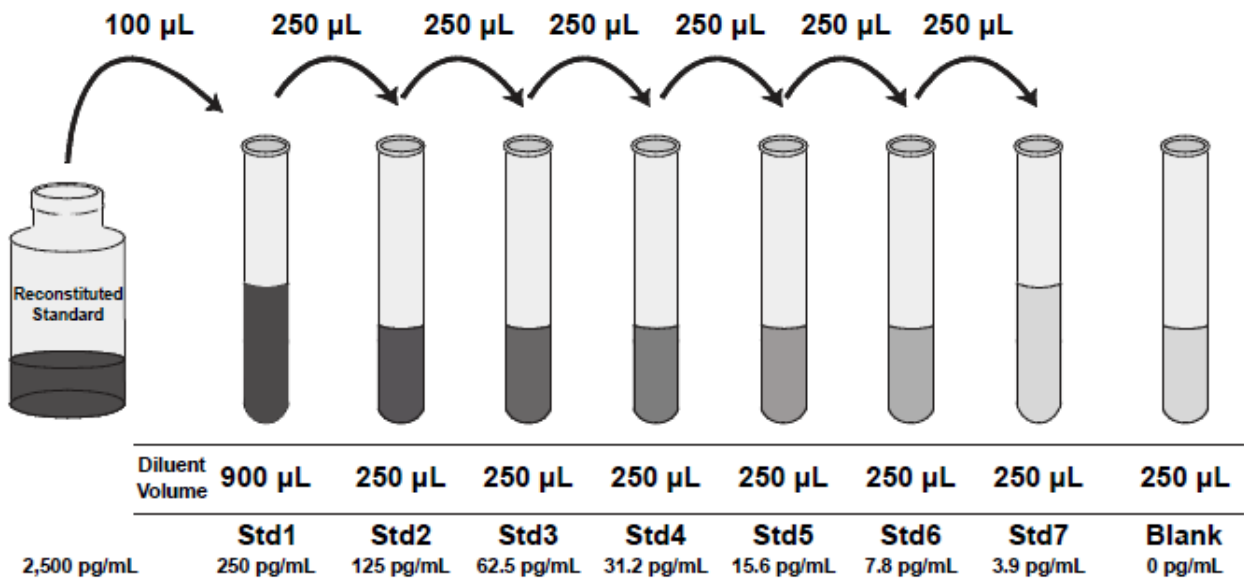
Perform sample dilutions with Standard Diluent Buffer.

### Dilute Standards

**Note:** Use glass or plastic tubes for diluting standards.

**Note:** This assay has been calibrated against the WHO reference preparation 86/680 (NIBSC, Hertfordshire, UK, EN6 3QG). One microgram equals 100,000 International Units.

1. Reconstitute Human IL-1 $\beta$  Standard to 2,500 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 2,500 pg/mL Human IL-1 $\beta$ . **Use the standard within 1 hour of reconstitution.**
2. Add 100  $\mu$ L Reconstituted Standard to one tube containing 900  $\mu$ L Standard Diluent Buffer and mix. Label as 250 pg/mL Human IL-1 $\beta$ .
3. Add 250  $\mu$ L Standard Diluent Buffer to each of 7 tubes labeled as follows: 125, 62.5, 31.2, 15.6, 7.8, 3.9, and 0 pg/mL Human IL-1 $\beta$ .
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



## Prepare 1X Streptavidin-HRP Solution

**Note:** Prepare 1X Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.



## ASSAY PROCEDURE

Total assay time: 2 hours and 55 minutes

Perform a standard curve with every assay.

Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

### Bind antigen

1. Add 50  $\mu$ L of standards, controls, or samples (see “Pre-dilute samples” on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
2. Add 100  $\mu$ L Hu IL-1 $\beta$  Biotin Conjugate solution into each well except the chromogen blanks.
3. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
4. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

### Add Streptavidin-HRP

1. Add 100  $\mu$ L 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
2. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
3. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

### Add Stabilized Chromogen

1. Add 100  $\mu$ L Stabilized Chromogen to each well. The substrate solution begins to turn blue.
2. Incubate for 25 minutes at room temperature in the dark.

**Note:** TMB should not touch aluminum foil or other metals.

## Add Stop Solution

Add 100  $\mu$ L Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

## Read Plate and Generate Standard Curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

## TYPICAL RESULTS

Standard curve example: The following data were obtained for the various standards over the range of 0-250 pg/mL human IL-1 $\beta$ .

Standard Human IL-1 $\beta$ (pg/mL)	Optical Density
250	2.65
125	1.51
62.5	0.95
31.2	0.50
15.6	0.35
7.8	0.24
3.9	0.15
0	0.09

## PERFORMANCE CHARACTERISTICS

Inter-assay precision: Samples were assayed 24 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	193.8	101.8	56.3
Standard Deviation	13.4	6.1	4.1
% Coefficient of Variation	6.9	6.0	7.3

Intra-assay precision: Samples of known human IL-1 $\beta$  concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	194.9	101.6	60.2
Standard Deviation	9.1	4.2	2.7
% Coefficient of Variation	4.7	4.1	4.5

## Linearity of Dilution

Pooled human serum containing 220 pg/mL of measured human IL-1 $\beta$  was serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

## Recovery

The recovery of human IL-1 $\beta$  added to pooled normal human serum or cell culture medium containing 1% and 10% fetal bovine serum was measured with the Human IL-1 $\beta$  ELISA Kit.

Sample	Range %	Average % Recovery
Serum	84-100	94
Cell culture medium	99-107	103

## **Sensitivity**

The analytical sensitivity of the assay is 1 pg/mL human IL-1 $\beta$ . This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times.

## **Specificity**

Buffered solutions of a panel of substances at 50 ng/mL were assayed with the Human IL-1 $\beta$  ELISA Kit. The following substances were tested and found to have no cross-reactivity: **human** IL-1 $\alpha$ , IL-1ra, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IFN- $\alpha$ , TGF- $\beta$ , GM-CSF, G-CSF, OSM, MIP-1 $\alpha$ , MIP-1 $\beta$ , LIF, MCP-1, G-CSF, RANTES, PF-4,  $\beta$ TG, GRO, IP-10, SCF, TNF- $\alpha$ , and TNF- $\beta$ .



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## NOTES



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