



## **TiterZyme<sup>®</sup> EIA**

### **rat IL-1 $\beta$**

# **Enzyme Immunometric Assay Kit**

**Catalog No. 900-131**

## **96 Well Kit**

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

## **Description**

Assay Designs' rat IL-1 $\beta$  TiterZyme<sup>®</sup> Enzyme Immunometric Assay (EIA) kit is a complete kit for the quantitative determination of rat IL-1 $\beta$  in biological fluids. Please read the complete kit insert before performing this assay. The kit uses an antibody to rat IL-1 $\beta$  immobilized on a microtiter plate to bind the rat IL-1 $\beta$  in the standards or sample. A recombinant rat IL-1 $\beta$  Standard is provided in the kit. After a short incubation, the excess sample or standard is washed out and a biotinylated antibody to rat IL-1 $\beta$  is added. This antibody binds to the rat IL-1 $\beta$  captured on the plate. After a short incubation the excess antibody is washed out and streptavidin conjugated to Horseradish peroxidase is added, which binds to the biotinylated rat IL-1 $\beta$  antibody. Excess conjugate is washed out and substrate is added. After a short incubation, the enzyme reaction is stopped and the color generated is read at 450 nm. The measured optical density is directly proportional to the concentration of rat IL-1 $\beta$  in either standards or samples. For further explanation of the principles and practices of immunoassays please see the excellent books by Chard<sup>1</sup> or Tijssen<sup>2</sup>.

## **Introduction**

Interleukin-1 (IL-1) is a family of related proteins that are considered to act as the prototypic multifunctional cytokine. Consisting of IL-1 $\alpha$ , IL-1 $\beta$ , and IL-1Ra (receptor antagonist), IL-1 elicits biological responses when present in lower pico- to femto-molar concentrations<sup>3</sup>. IL-1 $\beta$  is translated as a 31 kD precursor that is glycosylated and cleaved into a cytosolic pro-IL-1 $\beta$  molecule. The biologically active 17 kD fragment is released by enzymatic activity of the IL-1 $\beta$ -converting enzyme and secreted from the cell via ABC transporters<sup>4,5</sup>. IL-1 $\beta$  with the aid of inducible transcription factors initiates the expression of a pro-inflammatory cascade of proteins and cytokines by selective regulation of other molecules<sup>6</sup>. The broad range of regulatory events mediated by IL-1 $\beta$  include but are not limited to the induction of chemokines, suppression of constitutive housekeeping genes, initiation of Prostaglandin E<sub>2</sub> synthesis, the expression of inducible nitric oxide synthase, soluble PLA<sub>2</sub> and COX-2 as well as bone and cartilage remodeling<sup>3</sup>. IL-1 $\beta$  is expressed by a variety of cells including macrophages, monocytes, platelets and neutrophils. In addition, IL-1 $\beta$  production has been reported in NK cells, endothelial cells, fibroblasts, T cells, osteoblasts and a number of cells that comprise the central nervous system<sup>7</sup>. As the major pro-inflammatory cytokine, increased levels of IL-1 $\beta$  have been linked with rheumatoid arthritis, invasive malignancies, bacterial and viral infection, Alzheimer's Disease and congestive heart failure<sup>8-12</sup>.

## **Precautions**

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

1. Stop Solution is a 0.18 M sulfuric acid solution. This solution is caustic; care should be taken in use.
2. The activity of the Horseradish peroxidase conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine.
3. We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
4. The rat IL-1 $\beta$  Standard provided, Catalog No. 80-1259, should be handled with care because of the known and unknown effects of IL-1 $\beta$ .

### **Materials Supplied**

1. **rat IL-1 $\beta$  Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-1260**  
A plate using break-apart strips coated with antibody specific to rat IL-1 $\beta$ .
2. **rat IL-1 $\beta$  Antibody, 13 mL, Catalog No. 80-1257**  
A solution of biotinylated antibody to rat IL-1 $\beta$ .
3. **rat IL-1 $\beta$  Sample Diluent, 25 mL, Catalog No. 80-1258**
4. **rat IL-1 $\beta$  Streptavidin-HRP Concentrate, 75  $\mu$ L, Catalog No. 80-1256**  
A concentrated solution of Streptavidin conjugated to Horseradish peroxidase.
5. **rat IL-1 $\beta$  Streptavidin-HRP Dilution Buffer, 13 mL, Catalog No. 80-1255**
6. **Wash Buffer Concentrate, 50 mL, Catalog No. 80-1253**  
Tris buffered saline containing detergents.
7. **rat IL-1 $\beta$  Standard, 2 vials, Catalog No. 80-1259**  
Two vials of lyophilized recombinant rat IL-1 $\beta$ .
8. **rat IL-1 $\beta$  TMB Substrate, 12 mL, Catalog No. 80-1254**  
A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. Ready to use.  
**Protect from prolonged exposure to light.**
9. **rat IL-1 $\beta$  Stop Solution, 13 mL, Catalog No. 80-1261**  
A 0.18 M solution of sulfuric acid in water. Keep tightly capped. Caution: **Caustic.**
10. **rat IL-1 $\beta$  Assay Layout Sheet, 1 each, Catalog No. 30-0208**
11. **Plate Sealer, 3 each, Catalog No. 30-0012**

### **Storage**

All components of this kit are stable at 4 °C until the kit's expiration date.

### **Materials Needed but Not Supplied**

1. Deionized or distilled water. No difference in assay results is seen with distilled water.
2. Precision pipets for volumes between 100  $\mu$ L and 1,000  $\mu$ L.
3. Disposable polypropylene or polyethylene test tubes for dilution of samples and standards.
4. Repeater pipets for dispensing 50  $\mu$ L and 100  $\mu$ L.
5. Disposable beakers for diluting buffer concentrates.
6. Graduated cylinders.
7. Microcentrifuge to prepare Streptavidin-HRP solution.
8. Absorbent paper for blotting.
9. Microplate reader capable of reading at 450 nm, preferably with correction between 570 nm and 590 nm.
10. Graph paper for plotting the standard curve.

### **Sample Handling**

Assay Designs' TiterZyme<sup>®</sup> EIA is compatible with rat IL-1 $\beta$  samples in serum, plasma (EDTA, heparin and sodium citrate) and tissue culture media. Samples can be read directly from a standard curve diluted in the proper diluent.

Culture fluids, serum, and plasma are suitable for use in the assay. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples in the majority of tissue culture media can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Sample Diluent. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of rat IL-1 $\beta$  in the appropriate matrix.

If samples are to be run within 24 hours, they may be stored at 4 °C. Otherwise, samples must be stored frozen at -70 °C to avoid loss of bioactive rat IL-1 $\beta$ . Excessive freeze/thaw cycles should be avoided. Prior to assay, frozen sera should be brought to room temperature slowly and gently mixed by hand. Do not thaw samples in a 37 °C incubator. Do not vortex or sharply agitate samples.

### **Procedural Notes**

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards must be prepared in polypropylene or polyethylene tubes. Do not use polystyrene, polycarbonate, or glass tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses plates with removable strips. Unused wells must be kept desiccated at 4 °C in the sealed bag provided. The strips should be used in the frame provided.
8. **Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**
9. **It is important that the matrix for the standards and samples be as similar as possible. Rat IL-1 $\beta$  samples diluted with Sample Diluent should be run with a standard curve diluted in the same buffer. Serum and plasma samples should be evaluated against a standard curve run in Sample Diluent while Tissue Culture samples should be read against a standard curve diluted in the same complete but non-conditioned media. See Reagent Preparation, step #2.**

### **Reagent Preparation**

#### **1. Wash Buffer**

Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 1,450 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

#### **2. rat IL-1 $\beta$ Standards**

Reconstitute standard with deionized water. Reconstitution volume is stated on the standard vial label. Let it sit at room temperature for 5 minutes. Mix gently. This solution contains 5,000 pg/mL rat IL-1 $\beta$ . When testing serum or plasma samples, use the Sample Diluent provided to prepare standard curve serial dilutions. When using cell culture supernatants, use Tissue Culture Media to prepare the standard curve serial dilutions.

Label six 12x75 mm test tubes #1 through #6. Pipet 240  $\mu$ L of Sample Diluent or tissue culture media into tubes #1 through #6. Add 240  $\mu$ L of the 5,000 pg/mL Standard to tube #1. Vortex thoroughly. Add 160  $\mu$ L of tube #1 to tube #2 and vortex thoroughly. Add 160  $\mu$ L of tube #2 to tube #3 and vortex thoroughly. Continue this for #4 through #6.

**The concentration of rat IL-1 $\beta$  in tubes #1 through #6 will be 2,500, 1,000, 400, 160, 64 and 25.6 pg/mL respectively. See rat IL-1 $\beta$  Assay Layout Sheet for dilution details.**

**Diluted standards should be used within 60 minutes of preparation. Do not store reconstituted standards.**

### 3. Streptavidin-HRP Solution

Prepare Streptavidin-HRP solution **immediately before use**. Do not store prepared Streptavidin-HRP solution. Use a plastic tube to prepare Streptavidin-HRP solution. Briefly centrifuge the Streptavidin-HRP Concentrate to force entire vial contents to the bottom. For each strip used, mix 2.5  $\mu\text{L}$  of Streptavidin-HRP Concentrate with 1 mL of Streptavidin-HRP Dilution Buffer.

### Assay Procedure

**Bring all reagents to room temperature for at least 30 minutes prior to opening.**

**All standards, controls and samples should be run in duplicate.**

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4 °C.
2. Pipet 100  $\mu\text{L}$  of Sample Diluent or Tissue Culture Media into the S0 (0 pg/mL standard) wells.
3. Pipet 50  $\mu\text{L}$  of Sample Diluent or Tissue Culture Media into the Standard and Sample wells.
4. Pipet 50  $\mu\text{L}$  of Standards #1 through #6 into the appropriate wells.
5. Pipet 50  $\mu\text{L}$  of the Samples into the appropriate wells.
6. Tap the plate gently to mix the contents.
7. Seal the plate and incubate at room temperature for 2 hours.
8. Empty the contents of the wells and wash by adding 400  $\mu\text{L}$  of wash solution to every well. Repeat the wash 4 more times for a total of **5 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
9. Pipet 100  $\mu\text{L}$  of Antibody into each well, except the Blank.
10. Seal the plate and incubate at room temperature for 30 minutes.
11. Empty the contents of the wells and wash by adding 400  $\mu\text{L}$  of wash solution to every well. Repeat the wash 4 more times for a total of **5 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
12. Add 100  $\mu\text{L}$  of Conjugate to each well, except the Blank.
13. Seal the plate and incubate at room temperature for 30 minutes.
14. Empty the contents of the wells and wash by adding 400  $\mu\text{L}$  of wash solution to every well. Repeat the wash 4 more times for a total of **5 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
15. Pipet 100  $\mu\text{L}$  of Substrate Solution into each well.
16. Incubate for 30 minutes at room temperature in the dark.
17. Pipet 100  $\mu\text{L}$  Stop Solution to each well. This stops the reaction and the plate should be read immediately.
18. Blank the plate reader against the Blank wells, read the optical density at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

### **Calculation of Results**

Several options are available for the calculation of the concentration of rat IL-1 $\beta$  in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of rat IL-1 $\beta$  can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

$$\text{Average Net OD} = \text{Average OD} - \text{Average Blank OD}$$

2. Using linear graph paper, plot the Average OD for each standard versus rat IL-1 $\beta$  concentration in each standard. Approximate a straight line through the points. The concentration of rat IL-1 $\beta$  in the unknowns can be determined by interpolation.

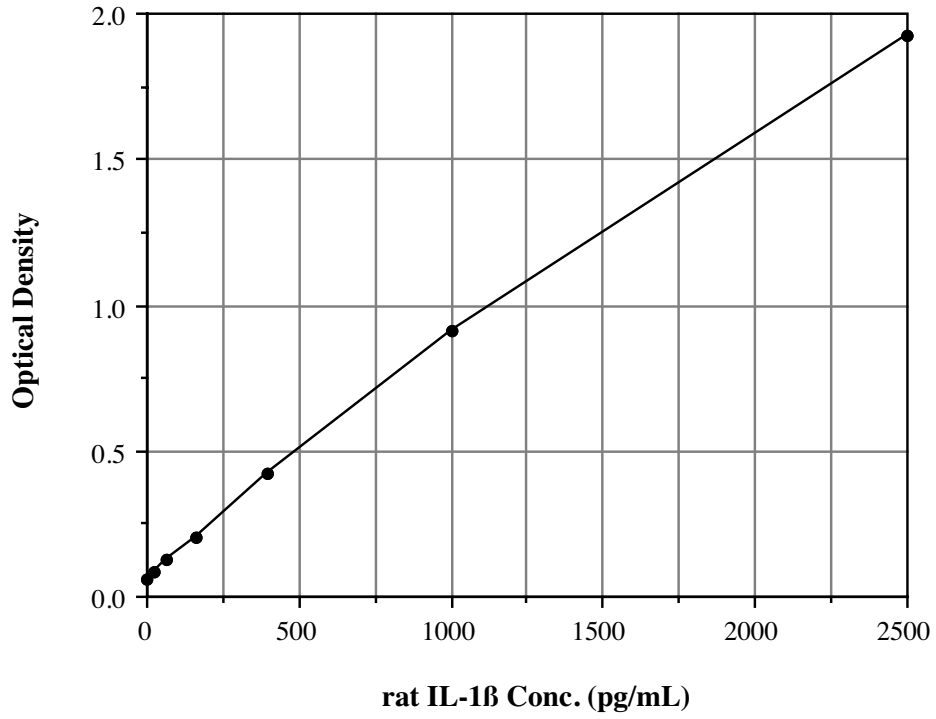
### **Typical Results**

The results shown below are for illustration only and **should not** be used to calculate results from another assay.

<u>Sample</u>	<u>Average OD</u>	<u>Net OD</u>	<b>rat IL-1<math>\beta</math> (pg/mL)</b>
Blank	(0.039)		
S0	0.097	0.058	<b>0</b>
S1	1.963	1.924	<b>2,500</b>
S2	0.951	0.912	<b>1,000</b>
S3	0.461	0.422	<b>400</b>
S4	0.245	0.206	<b>160</b>
S5	0.165	0.126	<b>64</b>
S6	0.121	0.082	<b>25.6</b>
Unknown 1	0.316	0.278	<b>235.8</b>

**Typical Standard Curve**

A typical standard curve is shown below. This curve **must not** be used to calculate rat IL-1 $\beta$  concentrations; each user must run a standard curve for each assay.



## **Performance Characteristics**

### **Sensitivity <12 pg/mL**

The sensitivity or Lower Limit of Detection (LLD) is determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

### **Linearity:**

A sample containing 1,911.8 pg/mL rat IL-1 $\beta$  was serially diluted 5 times 1:2 in the Sample Diluent supplied in the kit and measured in the assay. The data was plotted graphically as actual rat IL-1 $\beta$  concentration versus measured rat IL-1 $\beta$  concentration.

The line obtained had a slope of 0.9997 with a correlation coefficient of 0.9334.

### **Cross Reactivities**

The rat IL-1 $\beta$  TiterZyme<sup>®</sup> EIA Kit is specific for native and recombinant rat IL-1 $\beta$ . It is unaffected by the presence of rat IFN $\gamma$ , IL-1 $\alpha$ , IL-2, IL-4, IL-6, IL-10, MCP-1, MIP-1 $\alpha$ , or RANTES, or human, mouse or pig IL-1 $\beta$ .

## Sample Recoveries

Refer to pages 4 and 5 for Sample Handling recommendations and Standard preparation.

Recovery of rat IL-1 $\beta$  is evaluated using the Assay Designs' rat IL-1 $\beta$  TiterZyme<sup>®</sup> EIA Kit. Pooled serum and plasma samples from Wistar rats and sample diluent controls are spiked with recombinant rat IL-1 $\beta$ . Endogenous IL-1 $\beta$  levels are determined by evaluating non-spiked samples along with spiked aliquots of the same samples in the EIA. Expected values are calculated by adding endogenous IL-1 $\beta$  levels to those of spiked diluent controls. Percent (%) recovery is found by dividing observed by expected values. Results for representative individual samples and populations are reported below:

Sample	Level	Representative Sample			Sample Population			(n)
		Expected (pg/mL)	Observed (pg/mL)	Recovery (%)	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)	
Serum	1	935.8	939.2	100.4	935.8	898.7	96.0	5
	2	328.9	340.6	103.6	328.9	324.3	98.6	5
EDTA Plasma	1	935.8	883.2	94.4	935.8	905.0	96.7	4
	2	328.9	349.2	106.2	328.9	336.7	102.4	4
Citrate Plasma	1	1,006.7	977.4	97.1	1,006.7	949.6	94.3	5
	2	343.6	321.8	93.7	343.6	323.1	94.0	5
Heparin Plasma	1	1,006.7	986.1	98.0	1,006.7	987.6	98.1	4
	2	343.6	351.3	102.2	343.6	347.1	101.0	4

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## **LIMITED WARRANTY**

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Assay Designs must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if Assay Designs is not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.



**For more details concerning the information within this kit insert, or to order any of Assay Designs' products, please call (734) 668-6113 between 8:30 a.m. and 5:30 p.m. EST. Orders or technical questions can also be transmitted by fax or e-mail 24 hours a day.**

**Material Safety Data Sheet (MSDS) available on our website or by fax.**

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