Rat *IL-1β* ELISA

670.040.096 **1 x 96 tests**
670.040.192 **2 x 96 tests**

INTENDED USE

The rat **IL-1β** ELISA is an enzyme-linked immunosorbent assay for quantitative detection of rat **IL-1β** in cell culture supernatants, rat serum, plasma or other body fluids. **The rat IL-1β ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

SUMMARY

Interleukin-1 (IL-1), originally described in 1972 as lymphocyte activating factor (LAF) for its effects on thymocytes (1), is a polypeptide cytokine with two molecular forms. Both forms appear to mediate identical ranges of biological activity which include synthesis of the acute phase proteins by hepatocytes, chemotaxis of polymorphonucleocytes, and release of polymorphonucleocytes from blood and bone marrow (2). These effects coined the acronym leukocyte endogenous mediator (LEM). Early researchers also called IL-1β endogenous pyrogen, and it has been shown to induce fever (3) and is thought to contribute to wasting of muscles (PIF, proteolysis inducing factor) (4). Other activities associated with IL-1 are the induction of Prostaglandin E_2 by synovial cells and release of collagenase with resulting destruction of cartilage and bone resorption (catabolin, osteoclast activation factor) (5). In addition, IL-1, has multiple immunological functions including enhancement of IL-2 production by T cells and activation of B-cells (BAF) and thymocytes (6-8). A true pleiotrope, IL-1 may have tumoricidal activity via its release of IL-2 and interferon gamma and be indirectly antiviral by stimulating fibroblasts to release interferon beta (9, 10). In its role as mediator of sepsis, IL-1 has most recently been described as enhancing the growth of virulent E.coli (11).

PRINCIPLES OF THE TEST

An anti-rat IL-1β polyclonal coating antibody is adsorbed onto microwells.

Rat IL-1β present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated polyclonal anti-rat IL-1β antibody is added and binds to rat IFNγ captured by the first antibody.

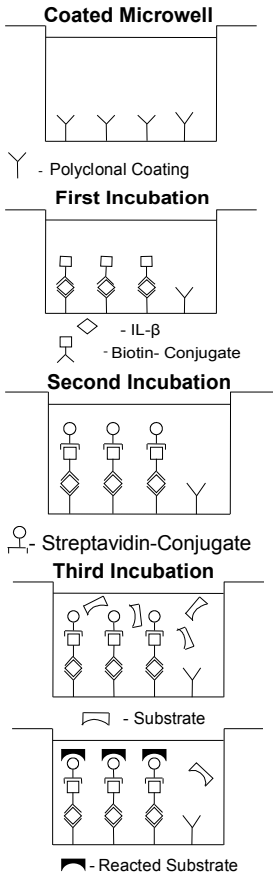
Following incubation unbound biotin conjugated anti-rat IL-1β is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-rat IL-1β.

Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of rat IL-1β present in the sample.

The reaction is terminated by addition of acid and absorbance is measured at 450 nm.

A standard curve is prepared from seven rat IL-1β standard dilutions and rat IL-1β sample concentration determined.



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REAGENTS PROVIDED

REAGENTS (store at 2-8°C)	QTY 1 plate	QTY 2 plates	RECONSTITUTION
96-wells precoated microtiter plate	1	2	Ready-to-use
Plate covers	4	8	
Rat IL-1β Standard	2 vials	4 vials	See label on the vial
Biotin-Conjugate anti-rat IL-1β polyclonal antibody*	1 vial	2 vials	(150 µl)
Assay Buffer Concentrate	1 vial	2 vials	(5 ml) 20X concentrate. Dilute in distilled water
Wash Buffer Concentrate	1 bottle	2 bottles	(50 ml) 20X concentrate. Dilute in distilled water
Substrate Solution	1 vial	2 vials	(15 ml) Ready-to-use
Streptavidin-HRP*	1 vial	2 vials	(150 µl)
Stop Solution (1 M Phosphoric acid)	1 vial	2 vials	(12 ml) Ready-to-use
Sample Diluent	1 bottle	2 bottles	(12 ml)
Blue Dye	1 vial	2 vials	(0.4 ml) Make a 1/250 dilution in the appropriate diluent
Green Dye	1 vial	2 vials	(0.4 ml) Make a 1/100 dilution in the appropriate diluent
Red Dye	1 vial	2 vials	(0.4 ml) Make a 1/250 dilution in the appropriate diluent

* It is recommended to spin vial in microcentrifuge before use to collect reagent at the bottom.

MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes
- 10 µl to 1,000 µl adjustable single channel micropipettes with disposable tips
- 50 µl to 300 µl adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

SAFETY

- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other 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 membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Reagents containing thimerosal as preservative may be toxic if ingested.
- Avoid contact of substrate solutions with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.
- Exposure to acids will inactivate the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solutions must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as if 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.

STORAGE INSTRUCTIONS

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.
The 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, the reagent is not contaminated by the first handling.

SPECIMEN COLLECTION, PROCESSING AND STORAGE

Cell culture supernatants, rat serum, plasma or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive rat IL-1β. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

For stability and suitability of samples refer to respective chapter.

PREPARATION OF REAGENTS

1. Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table.

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

2. Assay Buffer

Mix the contents of the bottle well. Add contents of **Assay Buffer Concentrate** (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days. Assay Buffer may be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

3. Preparation of Biotin-Conjugate

Make a 1:100 dilution of the concentrated **Biotin Conjugate** with **Assay Buffer** in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

4. Preparation of Standard

Reconstitute rat IL-1β **Standard** by addition of distilled water. Reconstitutions volume is stated on the label of the standard vial. Mix gently to ensure complete solubilization.

5. Preparation of Streptavidin-HRP

Make a 1:200 dilution of the concentrated **Streptavidin-HRP** solution in **Assay Buffer** as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (ml)
1 - 6	0.03	5.97
1 - 12	0.06	11.84

6. Addition of Colour-giving Dyes

In order to help our customers to avoid any mistakes in pipetting, DIACLONE now offers a new tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours to each step of the ELISA procedure.
This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.
Alternatively, the dye solutions from the stocks provided (***Blue-Dye, Green-Dye***) can be added to the reagents according to the following guidelines:

A. Diluent Buffer:

Before sample dilution add the ***Blue-Dye*** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of ***Blue-Dye***, proceed according to the instruction booklet.

5 ml Diluent	20 µl ***Blue-Dye***
12 ml Diluent	48 µl ***Blue-Dye***

B. Biotin-Conjugate:

Before dilution of the concentrated conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet, preparation of HRP-conjugate.

3 ml Assay Buffer	30 µl ***Green-Dye***
6 ml Assay Buffer	60 µl ***Green-Dye***

C. Streptavidin-HRP:

Before dilution of the concentrated Streptavidin-HRP, add the ***Red-Dye*** at a dilution of 1:250 (see table below) to the Assay Buffer (1x) used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the instruction booklet, preparation of Streptavidin-HRP.

6 ml Assay Buffer	24 µl ***Red-Dye***
12 ml Assay Buffer	48 µl ***Red-Dye***

TEST PROTOCOL

a. Prepare reagents immediately before use and mix them thoroughly without foaming.

b. Determine the number of Microwell Strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove sufficient **Microwell Strips coated with Monoclonal Antibody** (mouse) to rat IL-1β from their aluminium pouches immediately prior to use. Load them into the 96 microwell strip holder making sure to place the first microwell strip into row 1.

c. Wash the microwell strips twice with approximately 300 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.
After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

d. Add 100 µl of **Sample Diluent** in duplicate to all standard wells. Prepare standard dilutions by pipetting 100 µl of reconstituted (Refer to preparation of reagents) **rat IL-1β Standard**, in duplicate, into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100 µl to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of rat IL-1β standard dilutions ranging from 2000 to 31.25 pg/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of rat IL-1β standard dilutions:

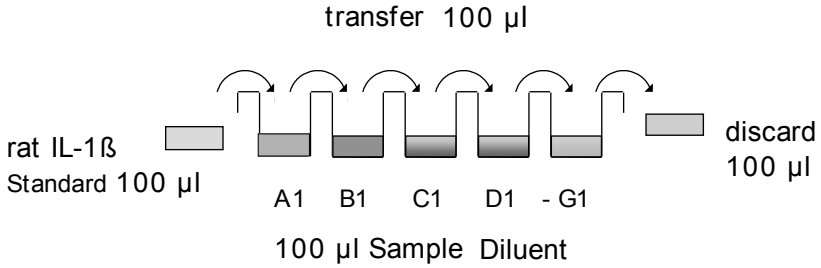


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	Standard Concentrations pg/mL		Sample wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	2000	2000										
B	1000	1000										
C	500	500										
D	250	250										
E	125	125										
F	62.5	62.5										
G	31.25	31.25										
H	Blank	Blank										

e. Add 100 µl of **Sample Diluent,** in duplicate, to the blank wells.

f. Add 50 µl of **Sample Diluent** to the sample wells.

g. Add 50 µl of each **Sample**, in duplicate, to the designated wells.

h. Prepare **Biotin-Conjugate** (Refer to preparation of reagents).

i. Add 50 µl of diluted **Biotin-Conjugate** to all wells, including the blank wells.

j. Cover with a **Plate Cover** and incubate at room temperature

(18° to 25°C) for 2 hours, if available on a microplate shaker set at 200 rpm.

k. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

l. Prepare **Streptavidin-HRP** (Refer to preparation of reagents).

m. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.

n. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 1 hour, if available on a microplate shaker at 200 rpm.

o. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

p. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.

q. Incubate the microwell strips at room temperature (18° to 25°C) for about **10 minutes** on a microplate shaker at 200 rpm. Avoid direct exposure to intense light. The point at which the substrate reaction need to be stopped is often determined by the ELISA reader being used. Many ELISA readers record absorbance only up to 2.0 O.D. **The colour development on the plate should be monitored and the substrate reaction stopped (see point s. of this protocol) before positive wells are no longer properly recordable.** It is recommended to add the Stop Solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as an OD of 0.6 – 0.65 is reached.

r. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

s. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the rat IL-1β standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the rat IL-1β concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating rat IL-1β for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding rat IL-1β concentration.

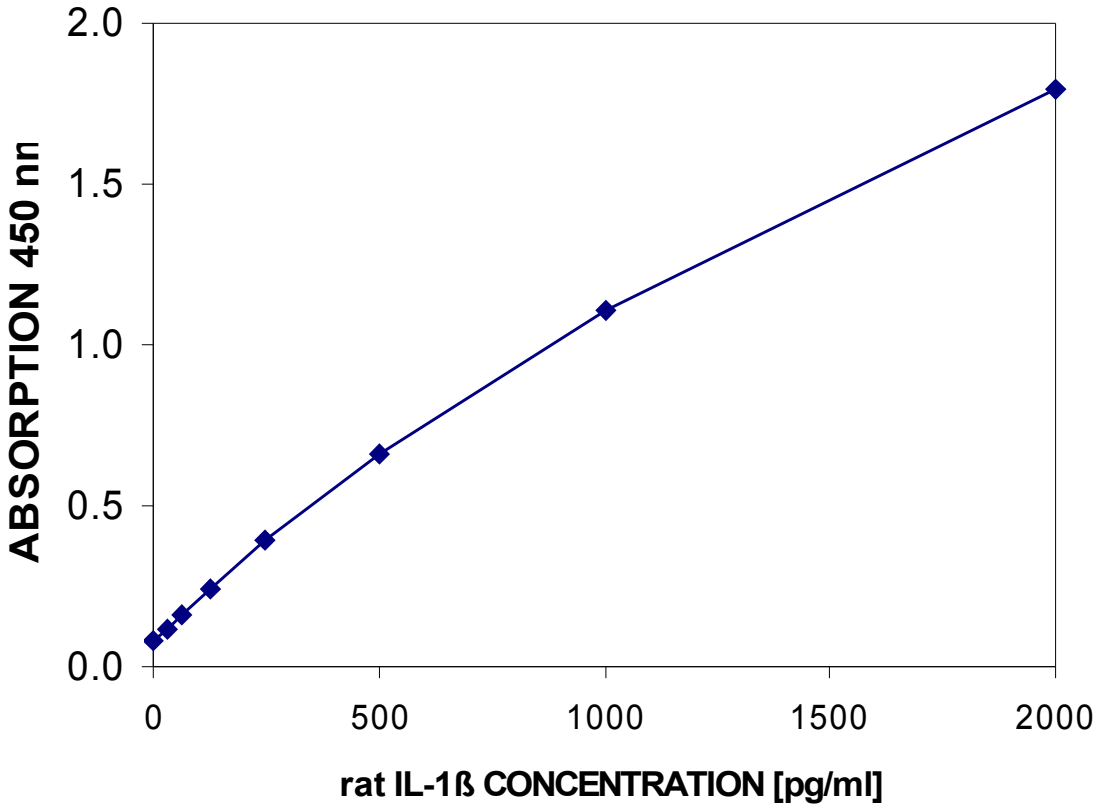
Samples have been diluted 1:2, thus the concentration read from the standard curve must be multiplied by the dilution factor (x2).

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low rat IL-1β levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual rat IL-1β level

- It is suggested that each testing facility establishes a control sample of known rat IL-1β concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

- A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for rat IL-1β ELISA. Rat IL-1β was diluted in serial two-fold steps in Sample Diluent, symbols represent the mean of three parallel titrations.
Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.



Typical data using the rat IL-1β ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	rat IL-1β Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	2000	1.814	1.797	0.9
	2000	1.780		
2	1000	1.119	1.112	0.7
	1000	1.104		
3	500	0.708	0.660	7.4
	500	0.611		
4	250	0.417	0.391	6.6
	250	0.365		
5	125	0.258	0.246	5.1
	125	0.233		
6	62.5	0.174	0.164	6.4
	62.5	0.153		
7	31.3	0.124	0.117	6.4
	31.3	0.109		
Blank		0.079	0.077	
		0.074		

LIMITATIONS OF THE PROCEDURE

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

PERFORMANCE CHARACTERISTICS

Sensitivity
The limit of detection of rat IL-1β defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 4.4 pg/ml (mean of 6 independent assays).

Reproducibility
a. Intra-assay
Reproducibility within the assay was evaluated in independent experiments. The overall intra-assay coefficient of variation has been calculated to be <10%.

b. Inter-assay
Assay to assay reproducibility within one laboratory was evaluated in independent experiments by three technicians. The overall inter-assay coefficient of variation has been calculated to be <10%.

Specificity
The interference of circulating factors of the immune systems was evaluated by spiking these proteins at physiologically relevant concentrations into a rat IL-1β positive serum. There was no detectable cross reactivity.

Spike Recovery
The spike recovery was evaluated by spiking four levels of rat IL-1β into pooled normal rat serum. Recoveries were determined in two independent experiments with 4 replicates each. Observed values showed an overall mean recovery of 88%.

Dilution Linearity
Rat serum spiked with different levels of rat IL-1β was assayed at four serial twofold dilutions with 4 replicates each. Experiments showed an overall mean recovery of 98 %.

Sample Stability
a. Freeze-Thaw Stability
Aliquots of spiked serum were stored frozen at –20°C and thawed up to 5 times, and rat IL-1β levels determined. There was no significant loss of IL-1β by freezing and thawing up to 5 times.

b. Storage Stability
Aliquots of spiked serum were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the rat IL-1β level determined after 24 h. There was no significant loss of rat IL-1β immunoreactivity during storage at -20°C, 4°C, room temperature and 37°C.

Expected Serum Values
There are no detectable rat IL-1β levels found in healthy rats. Elevated rat IL-1β levels depend on the type of immunological disorder.

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REAGENT PREPARATION SUMMARY

A. Wash Buffer

Add **Wash Buffer Concentrate** 20 x (50 ml) to 950 ml distilled water

B. Assay Buffer

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water(ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. Biotin-Conjugate

Make a 1:100 dilution according to the table.

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. Standard

Reconstitute rat IL-1β Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial.

E. Streptavidin-HRP

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (ml)
1 - 6	0.03	5.97
1 - 12	0.06	11.84

TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Sample Diluent**, in duplicate, to all standard wells
- Pipette 100 µl diluted **rat IL-1β Standard** into the first wells and create standard dilutions ranging from 2000 to 31.25 pg/ml by transferring 100 µl from well to well. Discard 100 µl from the last wells.
- Add 100 µl **Sample Diluent**, in duplicate, to the blank wells
- Add 50 µl **Sample Diluent**, in duplicate, to the sample wells
- Add 50 µl **Sample**, in duplicate, to designated wells
- Prepare **Biotin-Conjugate**
- Add 50 µl of diluted **Biotin-Conjugate** to all wells
- Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C), if available on a microplate shaker
- Prepare **Streptavidin-HRP**
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of diluted **Streptavidin-HRP** to all wells
- Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C), if available on a microplate shaker
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for about 10 minutes at room temperature (18°to 25°C), if available on a microplate shaker
- Add 100 µl **Stop Solution** to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low rat IL-1β levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual rat IL-1β level.