

Code No. 27194

## Rat TNF- $\alpha$ Assay Kit - IBL

### INTRODUCTION

TNF- $\alpha$  (Tumor Necrosis Factor-alpha) is a 17.5 kDa, 157 amino acids protein that is a potent lymphoid factor, which exerts cytotoxic effects on a wide range of tumor cells and other target cells. TNF- $\alpha$  has been suggested to play a pro-inflammatory role and has been detected in synovial fluids of patients with rheumatoid arthritis. It is the primary mediator of immune regulation. The biosynthesis of TNF- $\alpha$  is tightly controlled being produced in extremely small quantities in quiescent cells, but is a major secreted factor in activated cells.

### PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Rat TNF- $\alpha$ .

### MEASUREMENT RANGE

56.25 ~ 3,600 pg/mL

### INTENDED USE

- This IBL's assay kit is capable for the quantitative determination of Rat TNF- $\alpha$  in cell culture supernatant.
- Both recombinant and native forms of Rat TNF- $\alpha$  can be detected.

### KIT COMPONENT

1	Precoated plate: Anti-Rat TNF- $\alpha$ (C) Rabbit IgG Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Rat TNF- $\alpha$ Rabbit IgG Fab' Affinity Purify	0.4mL x 1
3	Standard : Recombinant Rat TNF- $\alpha$	1.0mL x 2
4	EIA buffer*	30mL x 1
5	Solution for Labeled antibody*	12mL x 1
6	Chromogen : TMB solution	15mL x 1
7	Stop solution*	12mL x 1
8	Wash buffer Conc.*	50mL x 1

### OPERATION MANUAL

#### 1. Materials needed but not supplied

- Plate reader (450nm)
- Graduated cylinder and beaker
- Refrigerator (as 4°C)
- Paper towel
- Plate washer or washing bottle\*
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
- Micropipette and tip
- Deionized water
- Graph paper (log/log)
- Tube for dilution of Standard

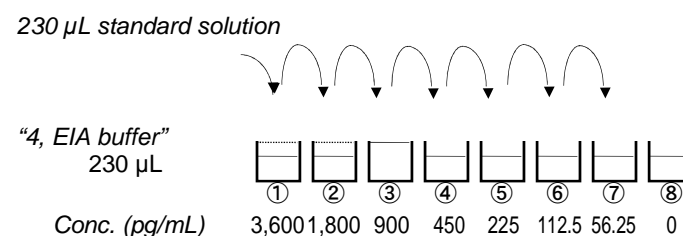
#### 2. Preparation

- 1) Preparation of wash buffer  
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
- 2) Preparation of Labeled antibody  
"2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.  
Example)  
In case you use one strip (8 well), the required quantity of Labeled antibody is 800  $\mu$ L. (Dilute 30  $\mu$ L of "2, Labeled antibody Conc." with 870  $\mu$ L of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100  $\mu$ L in each well.)  
This operation should be done just before the application of Labeled antibody.  
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
- 3) Preparation of Standard  
Put just 1.0 mL of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 7,200 pg/mL Rat TNF- $\alpha$  standard.
- 4) Dilution of Standard  
Prepare 8 tubes for dilution of "3, Standard". Put 230  $\mu$ L each of "4, EIA buffer" into the tube.  
Specify the following concentration of each tube."

Tube-1	3,600 pg/mL
Tube-2	1,800 pg/mL
Tube-3	900 pg/mL
Tube-4	450 pg/mL
Tube-5	225 pg/mL
Tube-6	112.5 pg/mL
Tube-7	56.25 pg/mL
Tube-8	0 pg/mL (Test Sample Blank)

Put 230  $\mu$ L of Standard solution into tube-1 and mix it gently. Then, put 230  $\mu$ L of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 3,600 pg/mL and 56.25 pg/mL. Tube-8 is the test sample blank as 0 pg/mL.

See following picture.



#### 5) Dilution of test sample

Test sample should be diluted with "4, EIA buffer" as necessary.

If the concentration of Rat TNF- $\alpha$  in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

### 3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
Test sample	100 $\mu$ L	Diluted standard (Tube 1-7) 100 $\mu$ L	EIA buffer (Tube-8) 100 $\mu$ L	EIA buffer 100 $\mu$ L
Incubation overnight at 4°C with plate lid				
4 times (wash buffer more than 350 $\mu$ L) (Refer to No. 8 and 9 described in OPERATING PRECATION.)*				
Labeled Antibody	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L	-
Incubation for 30 minutes at 4°C with plate lid				
5 times (wash buffer more than 350 $\mu$ L) (Refer to No. 8 and 9 described in OPERATING PRECATION.)*				
Chromogen	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L	100 $\mu$ L
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

- 1) Determine wells for reagent blank. Put 100  $\mu$ L each of "4, EIA buffer" into the wells.
- 2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100  $\mu$ L each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
- 3) Incubate the precoated plate overnight at 4°C after covering it with plate lid.
- 4) Washing (Refer to No. 8 and 9 described in OPERATING PRECATION.)\*  
Wash the plate with the prepared wash buffer and remove all liquid.\*
- 5) Pipette 100  $\mu$ L of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
- 6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
- 7) Washing (Refer to No. 8 and 9 described in OPERATING PRECATION.)\*  
Wash the plate with the prepared wash buffer and remove all liquid.\*
- 8) Take the required quantity of "6, Chromogen" into a disposable test tube. Then, pipette 100  $\mu$ L from the test tube into the wells. Please do not return the rest of the test tube to "6, Chromogen" bottle to avoid contamination.
- 9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by addition of "6, Chromogen".
- 10) Pipette 100  $\mu$ L of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by addition of "7, Stop solution".
- 11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank. The measurement shall be done within 30 minutes after addition of "7, Stop solution".

### OPERATING PRECATION\*

- 1) Test samples should be measured soon after collection. For storage of samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
- 2) Test samples should be diluted with "4, EIA buffer" contained in this kit.
- 3) Duplicate measurement of test samples and standards is recommended.
- 4) Standard curve should run for each assay.
- 5) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
- 6) All reagents should be brought to room temperature (R.T.) and mixed completely and gently before use. After mixing them, make sure of no change in quality of the reagents.
- 7) Use only "8, Wash buffer conc." contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
- 8) Using a plate washer is recommended (wait time zero second). It should be washed by a plate washer immediately after each reaction. If you use a washing bottle instead of a plate washer, after filling wash buffer in each well, immediately turn the plate upside down and shake it off to completely remove the wash buffer. Repeat the number of times of wash defined in a table for

measurement procedure described in section 3. It should be properly washed off as instructed in order to avoid any insufficient wash.

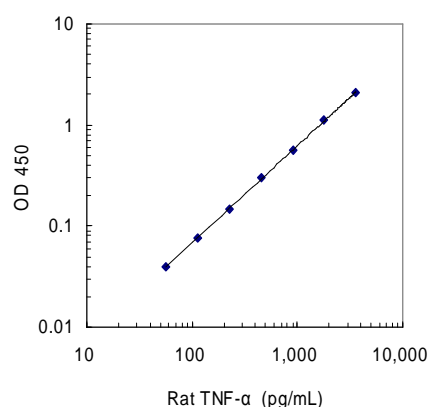
- 9) Carefully tap the plate against a clean paper towel without contacting with inside of each well to completely remove the washing buffer after repeated the determined number of wash.
- 10) "6, Chromogen - TMB solution" should be stored in the dark due to its sensitivity against light. It should be also avoided contact with metals. Required quantity should be prepared into a collecting container for each use.
- 11) After adding TMB solution into the wells, the liquid in the wells gradually changes the color in blue. In this process the plate should be in dark. Remained TMB solution in the collecting container should not be returned into the original bottle of TMB solution to avoid contamination.
- 12) Measurement of O.D. should be done within 30 minutes after addition of "7, Stop solution".

#### CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

Conc. (pg/mL)	Absorbance (450nm)
3,600	2.133
1,800	1.150
900	0.617
450	0.347
225	0.193
112.5	0.121
56.25	0.085
0 (Test Sample Blank)	0.046



\* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

#### PERFORMANCE CHARACTERISTICS

##### 1. Titer Assay (Samples with standard added are used.)

Specimen	Titer (X)	Measurement Value (pg/mL)	Theoretical Value (pg/mL)	%
10%FCS added RPMI-1640	2	1,391.64	1,600	87.0
	4	796.28	800	99.5
	8	409.48	400	102.4

##### 2. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measurement Value (pg/mL)	%
10%FCS added RPMI-1640 (x4)	1,800	1,555.68	86.4
	900	740.72	82.3
	450	380.54	84.6

##### 3. Intra - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
1,490.17	64.55	4.3	24
356.74	15.53	4.4	24
119.29	7.79	6.5	24

##### 4. Inter - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
1,435.88	90.95	6.3	12
346.76	22.49	6.5	12
112.03	11.39	10.2	12

#### 5. Specificity

Compound	Cross Reactivity
Rat TNF- $\alpha$	100 %
Rat IL-6	$\leq$ 0.1 %
Rat MCP-1	$\leq$ 0.1 %
Rat Osteopontin	$\leq$ 0.1 %
Rat CINC-1	$\leq$ 0.1 %

#### 6. Sensitivity

10.55 pg/mL

The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

#### PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. "3, Standard" is lyophilized products. Be careful to open this vial.
3. "7, Stop solution" is a strong acid substance. Therefore, be careful not to have your skin and clothes contact "7, Stop solution" and pay attention to the disposal of "7, Stop solution".
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in "2, Labeled antibody Conc.", however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

#### STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C

The expiry date is specified on outer box

Version 4.

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