

Code No. 27197

Rat IL-6 (Native Form) Assay Kit - IBL**INTRODUCTION**

Interleukin-6 (IL-6) is a cytokine that was discovered as a B-cell differentiation factor. IL-6 is not only an antibody-producing system, but its physiological activities, such as induction of acute-phase protein synthesis in hepatocytes, and growth stimulation based on its synergistic effect with IL-3, etc., have attracted great interest. Associations between IL-6 and pathology have been steadily demonstrated. For example, the growth factor in several diseases has been reported to be IL-6, and myeloma cells themselves have been reported to synthesize IL-6 and express IL-6 receptors. In addition, IL-6 has been suggested to contribute to a variety of inflammatory diseases and autoimmune diseases.

This kit makes it possible to detect the native form of IL-6 in rat blood and in the supernatant of cultured cells with high sensitivity.

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 IL-6 (Native Form).

MEASUREMENT RANGE

11.72 to 750 AU/mL (equivalent to Mouse IL-6, 8.36-535pg/mL)

The notation of the standard substance in this kit was decided by the unit notation, with its biological activity determined by bioassay as the index. More specifically, the ED₅₀ concentration in a cell growth test on a rat IL-6-dependent cell line was defined as 1 AU/mL.

Direct comparison of the data is not recommended, because reactivity with recombinant rat IL-6 expressed by *E.coli* and reactivity with the native form differ.

INTENDED USE

The IBL's Rat IL-6 (Native Form) Assay Kit is a complete kit for the quantitative determination of Rat IL-6 (Native Form) in EDTA-plasma and cell culture media.

KIT COMPONENT

1	Precoated plate : Anti- Rat IL-6 Rabbit IgG Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30 x)HRP conjugated Anti-Rat IL-6 Rabbit IgG Fab' Affinity Purify	0.4mL x 1
3	Standard : Recombinant Rat IL-6	0.5mL x 2
4	EIA buffer : 1% BSA, 0.05% Tween 20 in PBS	30mL x 1
5	Solution for Labeled antibody : 1% BSA, 0.05% Tween 20 in PBS	12mL x 1
6	Chromogen : TMB solution	15mL x 1
7	Stop solution : 1N H ₂ SO ₄	12mL x 1
8	Wash buffer Conc. : (40 x) 0.05% Tween20 in phosphate buffer	50mL x 1

OPERATION MANUAL**1. Materials needed but not supplied**

- Plate reader (450nm)
- Graduated cylinder and beaker
- Refrigerator (as 4°C)
- Paper towel
- Washing bottle for precoated plate
- 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, Wash buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50mL of "8, Wash buffer Conc." with 1,950mL 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 slit (8 well), the required quantity of Labeled antibody is 800 μL. (Dilute 30 μL of "2, Labeled antibody Conc." with 870 μL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 μL 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 0.5 mL of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 1,500 AU/mL Rat IL-6 (Native Form) standard.

4) Dilution of Standard

Prepare 8 tubes for dilution of "3, Standard". Put 230 μL each of "4, EIA buffer" into the tube.

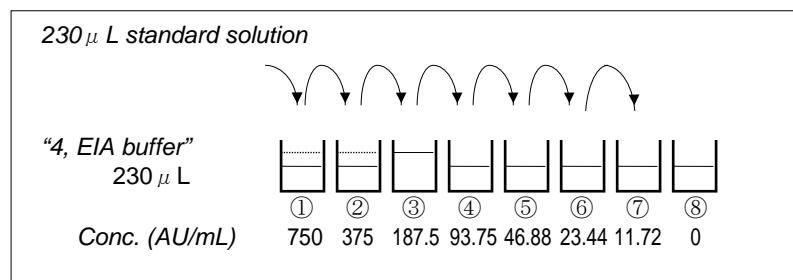
Specify the following concentration of each tube.

Tube-1	750 AU/mL
Tube-2	375 AU/mL
Tube-3	187.5 AU/mL
Tube-4	93.75 AU/mL

Tube-5	46.88 AU/mL
Tube-6	23.44 AU/mL
Tube-7	11.72 AU/mL
Tube-8	0 AU/mL (Test Sample Blank)

Put 230 μL of Standard solution into tube-1 and mix it gently. Then, put 230 μL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 750 AU/mL and 11.72 AU/mL. Tube-8 is the test sample blank as 0 AU/mL.

See following picture.

**5) Dilution of test sample**

Test sample may be diluted with "4, EIA buffer" if the need arises.

If the concentration of Rat IL-6 (Native Form) 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 μL	Diluted standard (Tube 1~7) 100 μL	EIA buffer (Tube-8) 100 μL	EIA buffer 100 μL
Incubation overnight at 4°C with plate lid				
Washing 7 times				
Labeled Antibody	100 μL	100 μL	100 μL	-
Incubation for 30 minutes at 4°C with plate lid				
Washing 9 times				
Chromogen	100 μL	100 μL	100 μL	100 μL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 μL	100 μL	100 μL	100 μL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

- Determine wells for reagent blank. Put 100 μL each of "4, EIA buffer" into the wells.
- Determine wells for test sample blank, test sample and diluted standard. Then, put 100 μL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1~7) into the appropriate wells.
- Incubate the precoated plate overnight at 4°C after covering it with plate lid.
- Wash each well of the precoated plate vigorously with wash buffer using the washing bottle. Then, fill each well with wash buffer and leave the precoated plate lay for 15~30 seconds. Remove wash buffer completely from the precoated plate by snapping. This procedure must be repeated more than 7 times. Then, remove the remaining liquid from all wells completely by snapping the precoated plate onto paper towel.
In case of using a plate washer, after 4 times washing with plate washer, washing with above washing bottle must be repeated 3 times.
- Pipette 100 μL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
- Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
- Wash the precoated plate 9 times in the same manner as 4).
- "6, Chromogen" should be taken the required quantity into a disposable test tube. Then, pipette 100 μL from the test tube into the wells. Please do not return the rest of the test tube to "6, Chromogen" bottle to avoid contamination.
- Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by the addition of "6, Chromogen".
- Pipette 100 μL 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".
- 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 450nm against a reagent blank. The measurement shall be done within 30 minutes after the addition of "7, Stop solution".

SPECIAL ATTENTION

- Test samples should be measured soon after the collection. For storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at low temperature and mix them completely before measurement.
- Test samples should be diluted with "4, EIA buffer", if the need arises.
- Duplicate measurement of test samples and standard is recommended.
- Use test samples in neutral pH range. The contaminations of organic solvent

may affect the measurement.

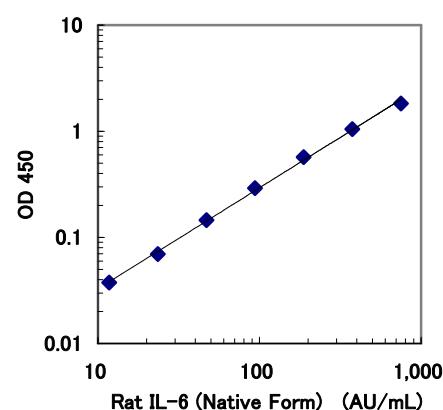
- 5) Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
- 6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
- 7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.
- 8) Measurement 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. (AU/mL)	Absorbance (450nm)
750	1.849
375	1.065
187.5	0.584
93.75	0.305
46.88	0.159
23.44	0.084
11.72	0.052
0 (Test Sample)	0.014



* 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 (AU/mL)	Theoretical Value (AU/mL)	%
10% FCS added RPMI-1640	2	383.96	375.00	102.4
	4	200.78	187.50	107.1
	8	106.19	93.75	113.3
Rat Plasma (EDTA)	8	74.55	93.75	79.5
	16	38.21	46.88	81.5
	32	19.07	23.44	81.4

2. Added Recovery Assay

Specimen	Theoretical Value (AU/mL)	Measurement Value (AU/mL)	%
10% FCS added RPMI-1640 (x2)	375.00	327.46	87.3
	187.50	174.79	93.2
	93.75	90.11	96.1
Rat Plasma (EDTA) (x16)	375.00	301.46	80.4
	187.50	152.21	81.2
	93.75	75.19	80.2

3. Intra - Assay

Measurement Value (AU/mL)	SD value	CV value (%)	n
297.31	4.94	1.7	24
71.71	1.39	1.9	24
34.83	0.80	2.3	24

4. Inter - Assay

Measurement Value (AU/mL)	SD value	CV value (%)	n
293.45	14.29	4.9	40
70.45	5.45	7.7	40
33.57	2.95	8.8	40

5. Specificity

Compound	Cross Reactivity
Rat IL-6 (Native Form)	100.0%
Rat GRO/CINC-1	—
Rat GRO/CINC-2 α	—
Rat GRO/CINC-2 β	—
Rat GRO/CINC-3	—
Rat MCP-1	—
Rat IL-1 β	—
Rat TNF α	—

6. Sensitivity

1.38 AU/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 different 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 1.