

## Mouse/Rat Intact Proinsulin CLEIA Kit - IBL

96 Well

Please read carefully this instruction prior you use this assay kit.

### INSTRUCTIONS FOR USE

This product is for research use only and is not intended for diagnostic use.

### KIT COMPONENT

1	<b>Precoated plate:</b> (Anti-Human Proinsulin 9F5 Mouse IgG MoAb.)	96Well x 1
2	<b>Labeled antibody conc.:</b> (50X) ALP conjugated Anti-Insulin 13G4m Mouse IgG Fab' A.P	0.15mL x 1
3	<b>Standard:</b> Recombinant Proinsulin (Synthetic peptide)	0.5mL x 1
4	<b>EIA buffer</b>	30mL x 1
5	<b>Solution for antibody</b>	12mL x 1
6	<b>Wash buffer conc.</b>	100mL x 1
7	<b>Chemiluminescent substrate</b>	6mL x 1
8	<b>Plate seal</b>	x 1

### MEASURING SAMPLES

Mouse Serum, EDTA-plasma and Heparin-Plasma  
Rat Serum, EDTA-plasma and Heparin-Plasma

### PRINCIPLE

This kit is a sandwich CLEIA (Chemiluminescent Enzyme Immunoassay). As a streptavidin is coated on a plate and biotinylated antibody is added into it to be fixed the capture antibody. Samples and standard are added into the wells for 1<sup>st</sup> reaction. After the reaction, ALP-conjugated secondary antibody is added into the wells for 2<sup>nd</sup> reaction. After washing away unbound the secondary antibody, Chemiluminescent substrate is added to the wells and measure the relative light units(RLU).

### OPERATING PRECATION

- 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.
- Test samples should be diluted with "4, EIA buffer" contained in this kit.
- Duplicate measurement of test samples and standards is recommended.
- Standard curve should run for each assay.
- Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
- 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.
- Use only "8, Wash buffer conc." contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
- 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.
- Unused wells are required to be protected by a plate cover sheet. Unused wells can be used at a later date.
- Measurement of chemiluminescence intensity should be conducted within 20 to 30 minutes after adding chemiluminescent substrate.

### OPERATION MANUAL AND DOSAGES

#### 1. Materials needed but not supplied.

Plate reader	Micropipette and tip
Test tubes for dilution	Measuring cylinder and beaker
Deionized water	Collecting container
Refrigerator	(i.e. clean disposable test tube)

#### 2. Preparation

##### (1) Preparation of wash buffer

Dilute "6, Wash buffer conc." 20 fold with deionized water. The diluted one is used for the assay as a wash buffer. Adjust the required quantities if needed.

##### (2) Preparation of labeled antibody

Dilute "2, Labeled antibody conc." 50 fold with "5, Solution for antibody" using a prepared collecting container.

##### Example)

In case you use one strip (8 well), the required quantity of Labeled antibody is 400  $\mu$ L. (Dilute 10  $\mu$ L of "2, Labeled antibody Conc." with 490  $\mu$ L of "5, Solution for labeled antibody" and mix it. And use 50 $\mu$ L the mixed solution in each well.)

This operation should be done just before applying labeled antibody.

The remaining "2, Labeled antibody Conc." should be stored at 4°C in a firmly sealed vial.

##### (3) Preparation of standard

Add 0.5 mL of deionized water into the vial of "3, Standard" and completely dissolve it. Concentration of the standard is 6,480 pg/mL. The standards enclosed in this kit can be frozen and stored after reconstitution. However the freeze-thaw shall not be repeated.

Prepare 6 test tubes for dilution of the standard and adding 200  $\mu$ L of the EIA buffer into each tube.

Put 100  $\mu$ L of 6,480 pg/mL standard into the tube 2,160 pg/mL (Tube-1) and gently mix it. Afterword, put 100  $\mu$ L of the mixed liquid of tube-1 into the tube 720 pg/mL (Tube-2) and gently mix it. Dilute two fold standard solution in series to set up 6 points of diluted standard between 6,480 pg/mL and 9 pg/mL.

Vial	6,480	pg/mL
Tube-1	2,160	pg/mL
Tube-2	720	pg/mL
Tube-3	240	pg/mL
Tube-5	80	pg/mL
Tube-5	27	pg/mL
Tube-6	9	pg/mL

### 3. Measurement Procedure

- Wash the plate with the prepared wash buffer and remove all liquid. (Refer to No. 8 described in OPERATING PRECATION.)
- Add 40  $\mu$ L "4, EIA Buffer" and 10  $\mu$ L prepared standard and samples into appropriate wells.
- Incubate with plate lid (1st reaction).
- Wash the plate with the prepared wash buffer and remove all liquid. (Refer to No. 8 described in OPERATING PRECATION.)
- Add 50  $\mu$ L prepared labeled antibody into the wells.
- Incubate with plate lid (2nd reaction).
- Wash the plate with the prepared wash buffer and remove all liquid completely. (Refer to No. 8 described in OPERATING PRECATION.)
- Add 50  $\mu$ L the chemiluminescent substrate into the wells.
- Incubate in dark
- Measurement of the relative light units(RLU)

Table for measurement procedure

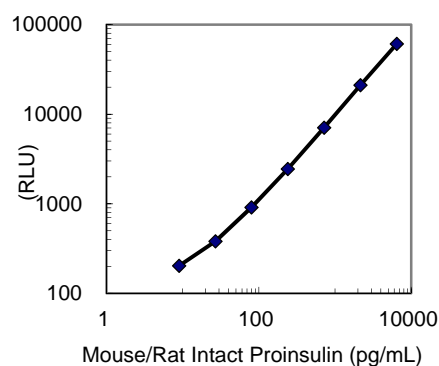
Sample	Test samples	Standard
Washing	3 times (wash buffer more than 350 $\mu$ L) (Refer to No. 8 described in OPERATING PRECATION.)	
EIA buffer	40 $\mu$ L	
Reagents	Test samples 10 $\mu$ L	Diluted Standard 10 $\mu$ L
1st reaction	Incubation for Overnight at 2 ~8°C with plate lid.	
Washing	5 times (wash buffer more than 350 $\mu$ L) (Refer to No. 8 described in OPERATING PRECATION.)	
Labeled antibody	50 $\mu$ L	
2nd reaction	Incubation for 120 minutes at room temperature with plate lid.	
Washing	5 times(wash buffer more than 350 $\mu$ L) (Refer to No. 8 described in OPERATING PRECATION.)	
Substrate	50 $\mu$ L	
Luminescent reaction	Incubation for 20 minutes at R.T. (shielded).	
Measuring (RLU)	Relative Light Units	

**CALCULATION OF TEST RESULT**

- Plot the concentration of the standard on the x-axis and its RLU on the y-axis. Draw a standard curve by applying appropriate regression curve on each plot (When analyzing software is used, spline curve is recommended.).
- Read the concentration by applying the RLU of the test samples on a standard curve.

Example of standard curve and measured value

Standard (pg/mL)	Luminous intensity (RLU)
6480	60,938
2160	21,118
720	7,063
240	2,443
80	911
27	382
9	203

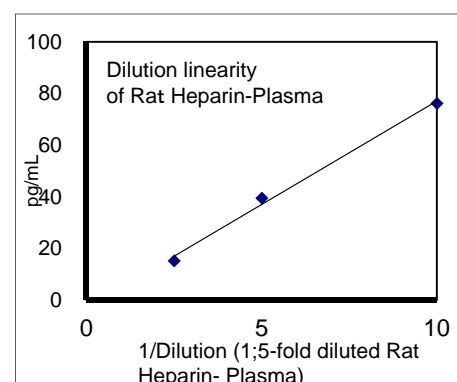
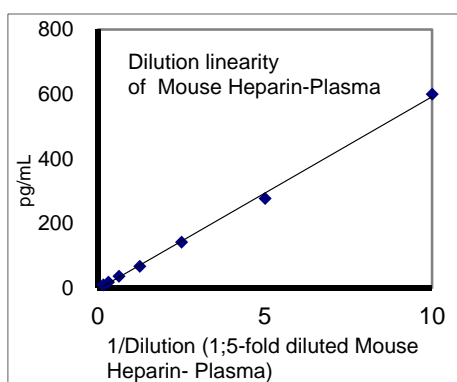
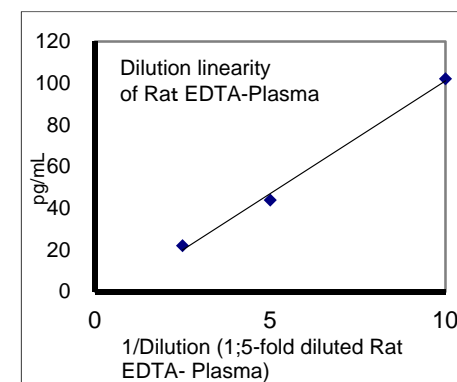
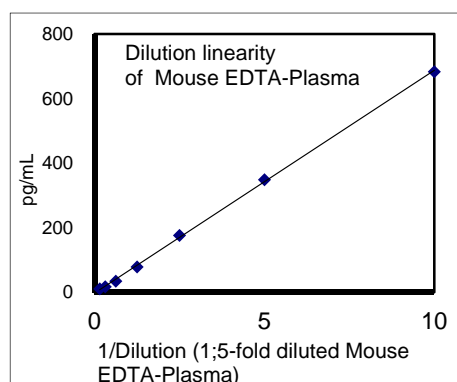
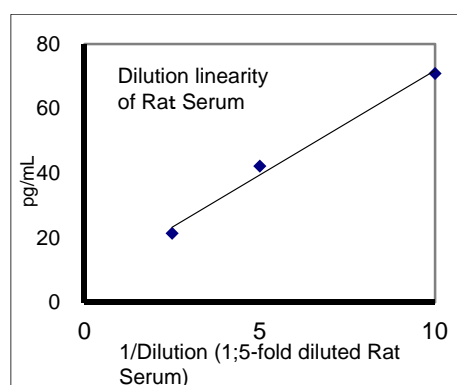
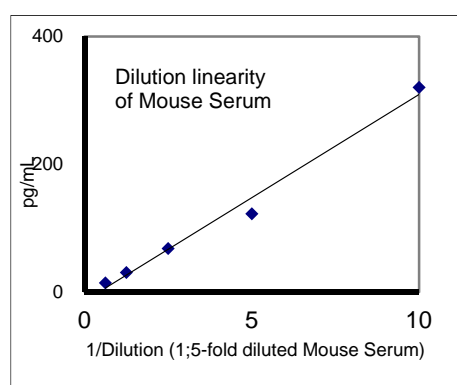


※Measured with Synergy HTX(Bio Tek Instruments)

**PERFORMANCE AND CHARACTERISTICS**

- Measurement range**  
9 ~ 6,480 pg/mL (1~ 720 pmol/L)

- Dilution linearity**



- Intra-assay**

Measurement value (pg/mL)	SD(pg/mL)	CV (%)	n
449.40	154.87	34.5	7
61.59	7.47	12.1	7

- Inter-assay**

Measurement value (pg/mL)	SD (pg/mL)	CV (%)	n
522.50	43.96	8.4	7
74.87	8.37	11.2	7

- Specificity**

Substance	Cross reactivity (%)
Mouse Insulin	0.1

- Interfering Substances**

Hemolyzed hemoglobin does not affect on the value of measurement up to 365 mg/dL.  
 Free bilirubin does not affect on the value of measurement up to 100 mg/dL.  
 Conjugated bilirubin does not affect on the value of measurement up to 100mg/dL.  
 Chyle does not affect on the value of measurement up to 7050 FTU.

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

- Precaution for handling (Hazard prevention)**

Treat the components carefully and wash hands after handling it.

- Precaution for intended use**

- "3, Standard" is lyophilized products. It should be careful to open this vial.
- All reagents should be stored at 2 - 8°C.
- Precipitation can be seen in "4, EIA buffer", "5, Solution for antibody" and "6, Wash buffer conc.", however, it does not affect its performance.
- Do not mix or replace the reagents with the reagents from a different lot or kit.
- Do not use expired reagents.

- Precaution for disposal**

- Dispose used materials after rinsing them with large quantity of water.

**STORAGE AND THE TERM OF VALIDITY**

Storage Condition: 2 - 8°C  
 The expiry date is specified on the outer box.

**PACKAGE UNIT AND PRODUCT NUMBER**

Package unit: 96 Well  
 Product number: 27708

**CONTACT DETAILS**

Immuno-Biological Laboratories Co., Ltd.  
 1091-1 Naka, Fujioka-Shi, Gunma 375-0005  
 TEL : 0274-22-2889  
 FAX : 0274-23-6055