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	**Precoated plate:** (Anti-Human Proinsulin 9F5 Mouse IgG MoAb.)	96Well x 1
2	**Labeled antibody conc.:** (50X) ALP conjugated Anti-Insulin 13G4m Mouse IgG Fab' A.P	0.15mL x 1
3	**Standard:** Recombinant Proinsulin (Synthetic peptide)	0.5mL x 1
4	**EIA buffer**	30mL x 1
5	**Solution for antibody**	12mL x 1
6	**Wash buffer conc.**	100mL x 1
7	**Chemiluminescent substrate**	6mL x 1
8	**Plate seal**	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 1st reaction. After the reaction, ALP-conjugated secondary antibody is added into the wells for 2nd reaction. After washing away unbound the secondary antibody, Chemiluminescent substrate is added to the wells and measure the relative light units(RLU). .

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 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.
9 Unused wells are required to be protected by a plate cover sheet. Unused wells can be used at a later date.
10 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 µL. (Dilute 10 µL of "2, Labeled antibody Conc." with 490 µL of "5, Solution for labeled antibody" and mix it. And use 50µL 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 µL of the EIA buffer into each tube.

Put 100 µL of 6,480 pg/mL standard into the tube 2,160 pg/mL (Tube-1) and gently mix it. Afterword, put 100 µL 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

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

Table for measurement procedure

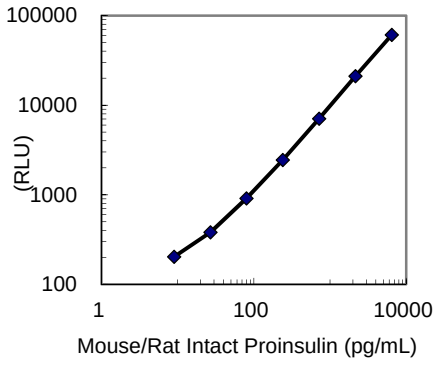
Sample	Test samples	Standard
Washing	3 times (wash buffer more than 350 µL) (Refer to No. 8 described in OPERATING PRECATION.)	
EIA buffer	40µL	
Reagents	Test samples 10 µL	Diluted Standard 10 µL
1st reaction	Incubation for Overnight at 2 ~8°C with plate lid.	
Washing	5 times (wash buffer more than 350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

CALCULATION OF TEST RESULT

1 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.).
2 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)	Luminouse intensity (RLU)
6480	60,938
2160	21,118
720	7,063
240	2,443
80	911
27	382
9	203

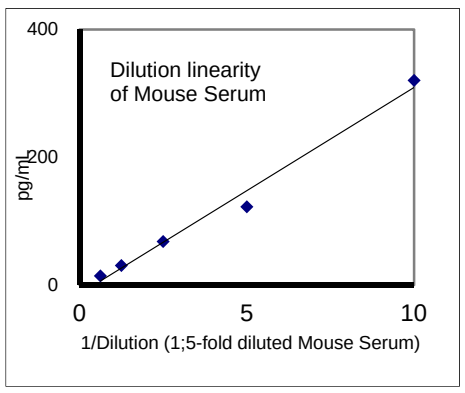


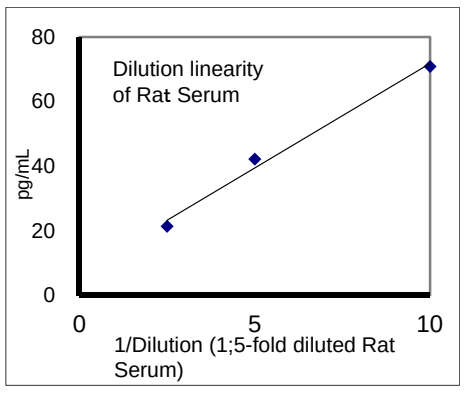
※Mesured with Synergy HTX(Bio Tek Instruments)

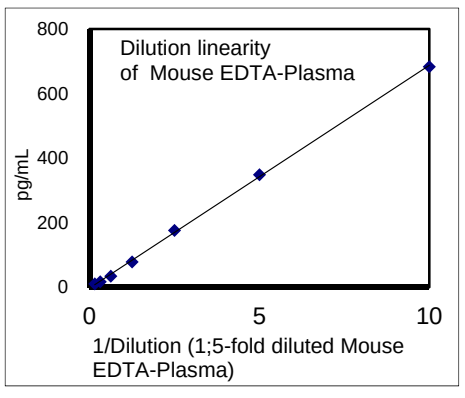
PERFORMANCE AND CHARACTERISTICS

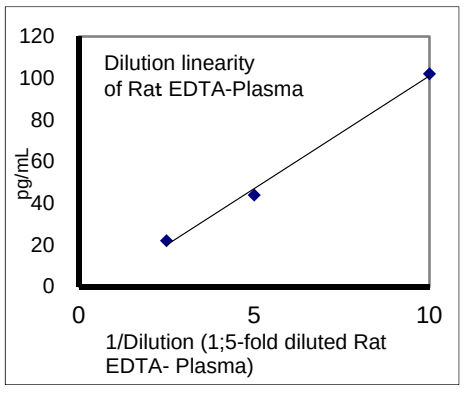
1 Measurement range
9 ～ 6,480 pg/mL (1~ 720 pmol/L)

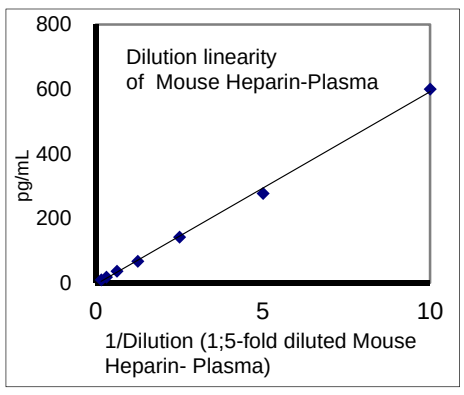
2 Dilution linearity

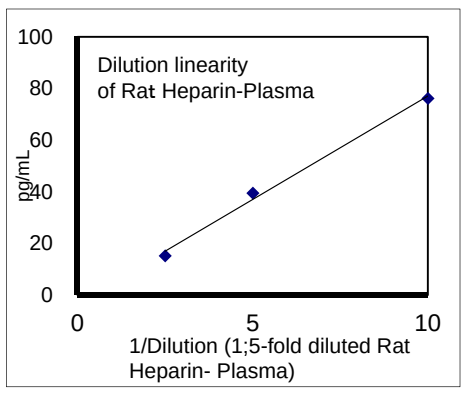












3 Intra-assay

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

4 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

5 Specificity

Substance	Cross reactivity (%)
Mouse Insulin	0.1

6 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

1 Precaution for handling (Hazard prevention)

Treat the components carefully and wash hands after handling it.

2 Precaution for intended use

(1) “3, Standard” is lyophilized products. It should be careful to open this vial.
(2) All reagents should be stored at 2 - 8°C.
(3) Precipitation can be seen in “4, EIA buffer”, “5, Solution for antibody” and “6, Wash buffer conc.”, however, it does not affect its performance.
(4) Do not mix or replace the reagents with the reagents from a different lot or kit.
(5) Do not use expired reagents.

3 Precaution for disposal

(1) 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

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