# Mouse/Rat Total Insulin 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

| | | |
|---|---|---|
| 1-1 | **Streptavidin plate** | 96Well x 1 |
| 1-2 | **Biotinylated Antibody:** Anti-Insulin 26B2m Mouse IgG Biotinylated | 6mL x 1 |
| 2 | **Labeled antibody conc.:** (50X) ALP conjugated Anti-Insulin 13G4m Mouse IgG Fab' A.P | 0.15mL x 1 |
| 3 | **Standard:** Recombinant Insulin | 1mL x 1 |
| 4 | **EIA buffer** | 30mL x 1 |
| 5 | **Solution for antibody** | 30mL x 1 |
| 6 | **Wash buffer conc.*** | 100mL x 1 |
| 7 | **Chemiluminescent substrate*** | 6mL x 1 |
| 8 | **Plate seal** | x 1 |

## MEASURING SAMPLES

Mouse and Rat EDTA-plasma and Serum

## 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 "6*, 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
Test tubes for dilution
Deionized water
Refrigerator
Plate shaker
Micropipette and tip
Measuring cylinder and beaker
Collecting container
(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 biotinylated antibody
Add 6mL of "5. Solution of antibody" into "1-2. Biotinylated antibody" and completely dissolve it. This solution is used as dissolved biotinylated antibody for measurement. The dissolved biotinylated antibody can be freeze stored. Freeze and thaw should not be repeated.

(3) Preparation of labeled antibody
Dilute "2, Labeled antibody conc." 50 fold with "5, Solution for antibody" using a prepared collecting container.

(4) Preparation of standard
Add 1 mL of deionized water into the vial of "3, Standard" and completely dissolve it. Concentration of the standard is 30,000 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 30,000 pg/mL standard into the tube 10,000 pg/mL (Tube-1) and gently mix it. Afterword, put 100 µL of the mixed liquid of tube-1 into the tube 3,333 pg/mL (Tube-2) and gently mix it. Dilute two fold standard solution in series to set up 6 points of diluted standard between 30,000 pg/mL and 41.15 pg/mL.

| | | | |
|---|---|---|---|
| Vial | 30,000 | pg/mL | (5,025 pmol/L) |
| Tube-1 | 10,000 | pg/mL | (1,675 pmol/L) |
| Tube-2 | 3,333.33 | pg/mL | (558 pmol/L) |
| Tube-3 | 1,111.11 | pg/mL | (186 pmol/L) |
| Tube-5 | 370.37 | pg/mL | (62 pmol/L) |
| Tube-5 | 123.46 | pg/mL | (21 pmol/L) |
| Tube-6 | 41.15 | pg/mL | (7 pmol/L) |

**3. Measurement Procedure**

(1) Add 50µL dissolved biotinylated antibody into each well.
(2) Coat the dissolved biotinylated antibody with plate lid and incubate.
(3) Wash the plate with the prepared wash buffer and remove all liquid. (Refer to No. 8 described in OPERATING PRECATION.)
(4) Add 45 µL "4. EIA Buffer" and 5 µL prepared standard and samples into appropriate wells. (Dilute standard and test samples 10 fold by the enclosed EIA buffer and add 50 µL each well if plate shaker is not used.)
(5) Shake with the plateshaker for 3 min and Incubate with plate lid (1st reaction).
(6) Wash the plate with the prepared wash buffer and remove all liquid. (Refer to No. 8 described in OPERATING PRECATION.)
(7) Add 50 µL prepared labeled antibody into the wells.
(8) Incubate with plate lid (2nd reaction).
(9) Wash the plate with the prepared wash buffer and remove all liquid completely. (Refer to No. 8 described in OPERATING PRECATION.)
(10) Add 50 µL the chemiluminescent substrate into the wells.
(11) Incubate in dark
(12) Measurement of the relative light units(RLU)

Table for measurement procedure

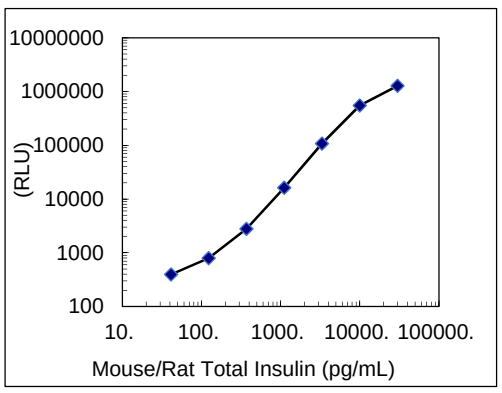
| | | |
|---|---|---|
| Dissolved biotinylated antibody | 50µL | |
| Coating | Incubation for 60 minutes at R.T. (shielded). | |
| Washing | 4 times (wash buffer more than 350 µL) (Refer to No. 8 described in OPERATING PRECATION.) | |
| EIA buffer | 45µL | |
| Sample | Test samples | Standard |
| Reagents | Test samples 5 µL | Diluted Standard 5 µL |
| 1st reaction | Shaking with plate shaker for 3min and 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 | 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 | 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) | Relative Light Unit |
|---|---|
| 30000. | 1,280,182 |
| 10000. | 549,100 |
| 3333.33 | 107,316 |
| 1111.11 | 16,205 |
| 370.37 | 2,793 |
| 123.46 | 797 |
| 41.15 | 396 |
| 0 | 286 |



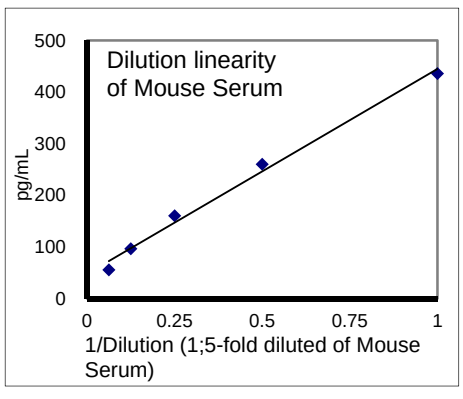

※Measured with Synergy HTX (Bio Tek Instruments)

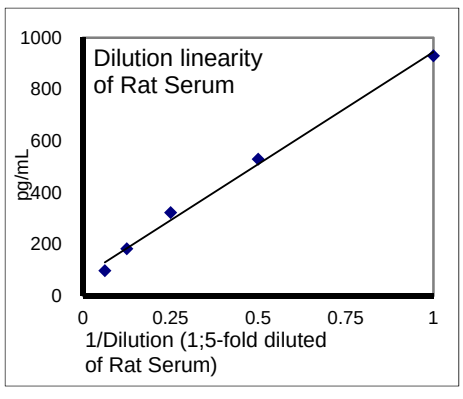
## PERFORMANCE AND CHARACTERISTICS

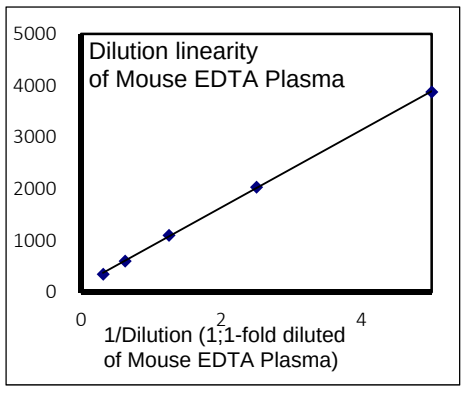
**1 Measurement range**

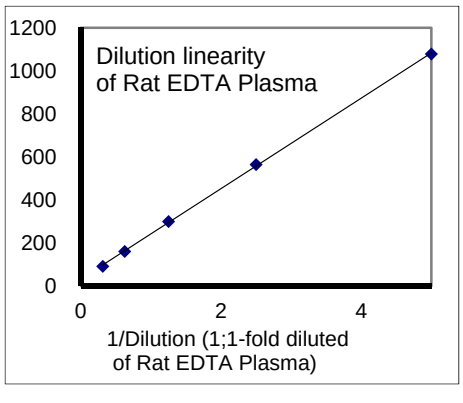
41.15 ～ 30,000 pg/mL (7 ~ 5,025 pmol/L)

**2 Dilution linearity**




**3 Added recovery assay**

| Specimen | Additive Amount (pg/mL) | Theoretical Value (pg/mL) | Measurement Value (pg/mL) | % |
|---|---|---|---|---|
| Mouse Serum | 15000. | 15446.62 | 13614.58 | 88.1% |
| | 5000. | 5446.62 | 4643.08 | 85.2% |
| | 1666.67 | 2113.28 | 1761.46 | 83.4% |
| Rat Serum | 15000. | 17038.98 | 16460.82 | 96.6% |
| | 5000. | 7038.98 | 6006.41 | 85.3% |
| | 1666.67 | 3705.65 | 3492.06 | 94.2% |
| Mouse EDTA-Plasma | 10000. | 11108.48 | 11802.92 | 106.3% |
| | 3333.33 | 4441.81 | 4125.73 | 92.9% |
| | 1111.11 | 2219.59 | 2011.43 | 90.6% |
| Rat EDTA-Plasma | 10000. | 10688.04 | 11139.53 | 104.2% |
| | 3333.33 | 4021.37 | 3599.36 | 89.5% |
| | 1111.11 | 1799.15 | 1441.83 | 80.1% |

**4 Intra-assay**

| Measurement value (pg/mL) | SD(pg/mL) | CV (%) | n |
|---|---|---|---|
| 5979.09 | 154.87 | 2.6 | 24 |
| 669.30 | 14.12 | 2.1 | 24 |
| 57.75 | 7.47 | 12.9 | 24 |

**5 Inter-assay**

| Measurement value (pg/mL) | SD (pg/mL) | CV (%) | n |
|---|---|---|---|
| 5734.27 | 301.73 | 5.3 | 6 |
| 655.45 | 31.87 | 4.9 | 6 |
| 69.79 | 6.69 | 9.6 | 6 |

**6 Interfering Substances**

Hemolyzed hemoglobin does not affect on the value of measurement up to 200 mg/dL.
Free bilirubin does not affect on the value of measurement up to 200 mg/dL.
Conjugated bilirubin does not affect on the value of measurement up to 240mg/dL.
Chyle does not affect on the value of measurement up to 14,100 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: 27707

## CONTACT DETAILS

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