Hamster insulin assay using Rat Insulin ELISA KIT

Research Reagent

For in vitro laboratory use only!

Please, read this instruction carefully before use.
This is an instruction for measurement of hamster insulin with high specificity and high sensitivity using Shibayagi's Rat Insulin ELISA KIT.

⚠ You need to purchase both Shibayagi's code# AKRIN-010T; **Rat Insulin ELISA KIT(TMB)** and code# ASIN-001; **Hamster Insulin Standard** in order to assay hamster insulin.
Rat Insulin ELISA KIT (TMB) is not included in Hamster Insulin Standard.

Advantage

(1) Rapid assay (total reaction time: 3 hours).
(2) A small sample volume (10µl in the standard procedure).
(3) An ecologically excellent preservative is used.
(4) Every reagent is provided in liquid form and ready to use.
(5) Excellent precision and reproducibility.
(6) A simple assay procedure without any pretreatment of samples.

Preparation

	Reagents	Amounts
(A)	Anti-rat insulin-coated plate	96 wells(8x12) / 1 plate
(B)	Standard rat insulin solution * (not for hamster!)	25µl / 1 vial
(C)	Buffer solution	60ml/1 bottle
(D)	Biotin-conjugated anti-rat insulin	10µl/ 1 vial
(E)	Peroxidase-conjugated streptavidin	20µl/ 1 vial
(F)	Chromogenic substrate reagent (TMB)	12ml/ 1 bottle
(H)	Reaction stopper (1M H_2SO_4)	12ml/ 1 bottle
(I)	Concentrated washing buffer(10x)	100ml/ 1 bottle

Assay sample

Hamster serum or plasma* 10µl in the standard procedure.
* We recommend to use heparin in obtaining plasma.

Purpose

Measurement of insulin in hamster

Assay range

0.156 ~ 10ng/ml in the standard procedure (sample volume 10µl)

Assay operation

1. Equipments necessary but not included in the kit.
(1) Micropipette (a micropipette able to deliver sample volume with high precision.),and a pipette for repetitive dispensing.
(2) Microplate washing apparatus (a microplate washer or a flashing bottle with nozzle).
(3) A microplate reader (A densitometer for microplate).

2. Preparation of reagents
(1) Washing buffer: Dilute the concentrated washing buffer (I) to 10X with purified water.
(2) Biotin-conjugated anti-insulin (D): Dilute to 4,000X with the buffer solution(C).

(3) HRP-conjugated streptavidin (E): Dilute to 2,000X with the buffer solution(C).
(4) Other reagents are used as they are.
(5) All the reagent solutions should be used after brought back to room temperature(20-25C).

3. An example of preparing standard solutions

Prepare the series of standard solutions starting from the original solution of **Hamster Insulin Standard Solution, 10ng/ml**, and serial dilution with the buffer solution as shown below.

Conc.(ng/ml)	10	5	2.5	1.25	0.625	0.313	0.156	0
Std. Sol.(µl)	Orig.sol.	Orig.sol:100	100*	100*	100*	100*	100*	0
Buffer (µl)	0	100	100	100	100	100	100	100

*One rank higher standard solution

4. Assay procedure

(1) Remove the cover sheet of the microplate after getting back to room temperature.
(2) Wash the anti-insulin coated plate (A) by filling the washing buffer and discard 4 times, then strike the plate upside-down onto folded several sheets of paper towel to remove buffer drops remaining in wells.
(3) Pipette 100µl of biotin-conjugated anti-insulin solution to all wells.
(4) Pipette 10µl of sample to sample-assay wells.
(5) Pipette 10µl of standard solution to the wells assigned for preparing a standard curve.
(6) Shake the plate gently on a plate shaker (800rpm for 10 seconds x 3 times).
(7) Incubate for 2 hour at room temperature (20-25oC).
(8) Discard the reaction mixture. Rinse wells by filling the washing buffer and discard 4 times, then strike the plate upside-down onto folded several sheets of paper towel to remove buffer drops remaining in wells.
(9) Pipette 100µl of HRP-conjugated avidin solution to all wells, and shake as (6).
(10) Incubate the plate for 30 minutes at room temperature.
(11) Discard the reaction mixture, and then wash the plate as (8).
(12) Pipette 100µl of chromogenic substrate solution (F) to wells, and shake as (3).
(13) Incubate the plate for 30 minutes at room temperature.
(14) Add 100 µl of the reaction stopper (H) to all wells and shake as (6).
(15) Measure the absorbance of each well at 450 nm (sub-wave length, 620nm) by a plate reader within 30 minutes.

Summary of Assay Procedure

Antibody-coated 96 well plate
↓
Washing 4 times
↓
Biotin-conjugated anti-insulin 100µl
↓ Shaking
Standard or sample 10µl
↓
Shaking and reaction for 2 hours at room temp.
↓
Washing 4 times
↓
Peroxidase-avidin conjugate 100µl
↓
Shaking and reaction for 30 mins. at room temp
↓
Washing 4 times
↓
Chromogenic substrate solution 100µl
↓
Shaking, and reaction for 30 mins. at room temp
↓
Reaction stopper (1M H_2SO_4) 100µl
↓
Shaking and measurement of absorbance
at 450nm(sub. 620nm)
Room temp.: 20~25C

Calculation of hamster insulin concentration

(1) Prepare a standard curve using semi-logarithmic or logarithmic section paper by plotting absorbance* (Y-axis) against insulin concentration (ng/ml) on X-axis.
*Absorbance at 450nm minus absorbance at 620nm.
(2) Using the standard curve, read the insulin concentration of a sample from its absorbance*, and multiply the assay value by dilution rate if the sample has been diluted. Though the assay range is wide enough, in case the absorbance of some samples are higher than that of the highest standard, please repeat the assay after proper dilution of samples with the buffer solution.
* We recommend the use of 3rd order regression curve or 4 parameter method in computer calculation.

Important notice in the treatments

1. Treatment of assay samples

(1) Use serum or plasma samples obtained by ordinary standard method.
Please, avoid using NaF-containing blood sampling tube, because fluoride ion is a peroxidase inhibitor, and may reduce the coloration even after washing.
(2) Turbid samples or those containing insoluble matters should be centrifuged before assay and use the clear supernatant fluid.
(3) Measure the samples as soon as possible after sampling.

2. Storage of assay samples.

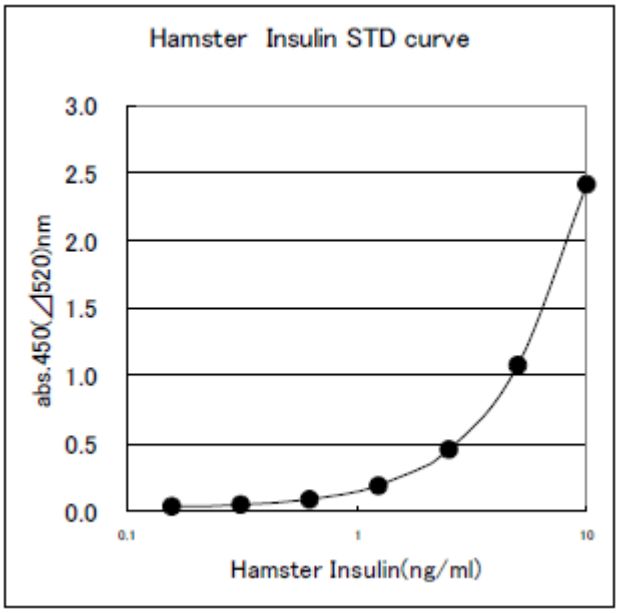
If assay samples have to be stored for a long period, freeze samples and store below -35C. Avoid repeated freezing and thawing.

3. Influence of interfering substances

If presence of interfering substances is suspected, examine by a dilution test using more than 2 points.

Assay range and assay data

Model standard curves



Assay data (by Shibayagi Co. Ltd.)

Animals: Armenian hamsters (8 weeks of age), males, fasted for 24 hours.
Assay samples: sera from 5 animals.
Assay system: Rat Insulin ELISA Kit (AKRIN-010T) with hamster insulin standard
Assay results: Mean: 0.441ng/ml , SD: 0.178ng/ml

Statements and precaution

(1) The reagents included in this assay kit should be used only for research works.
(2) The reagent solutions of the kit should be used principally immediately after reconstitution. Otherwise, keep them in a dark place with the temperature 2-8C,and use them within 3 days.
(3) The reagents were prepared to give accurate results by their combination within the kit. So, do not combine the reagents in the kit of other lot number. Even the lot number is the same, do not mix the reagents with those that have been preserved for some period.
(4) Pipetting and dilution of the reagent solutions should be made accurately because these steps influence the assay precision.
(5) Do not dry the assay plate to avoid denaturation of the coated antibody.
(6) Measurement of the reaction time should be started from the pipetting of reagent to the first well.
(7) Prepare the standard curve in each assay.
(8) Dilution of the assay sample must be carried out using the buffer solution attached to the kit.
(9) Storage condition for the kit should be strictly followed.
(10) Be careful not to allow the reagent solutions of the kit to touch the skin and mucus.
Especially be careful for the stopping solution because it is 1M sulfuric acid.
(11) HRP-conjugated reagent solution, chromogenic substrate solution, and reaction stopper must be avoided from contacting with any metal.
(12) In treating assay samples of animal origin, be careful for possible biohazards.
(13) As the antibody-coated plate is module type of 8wells x 12 rows, each row can be separated by a cutter and used independently.

Storage condition

Store the kit at 2~8C. Do not freeze.

Term of validity

Six months from production. Expiration date is indicated on the container.

Data sheet

Data sheet Link

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