Mouse Insulin ELISA Kit

For in-vitro laboratory use only!

Mouse Insulin ELISA Kit is a reagent kit for quantitation of Insulin by sandwich technique of enzyme immunoassay.

[**Advantage**]

(1)Mouse Insulin ELISA Kit is a high speed EIA. (for 3 ～ 4 hours).

(2)Mouse Insulin ELISA Kit can measure in samples (10μl) of very small volume.

(3)Mouse Insulin ELISA Kit　ensures simple assay procedures.

[**Reagents**]

A : Anti-Insulin-coated plate	96well(8x12)	x1
B : Standard Mouse Insulin solution (200ng/ml)	25μl	x1
C : Buffer solution	60ml	x1
D : Biotin conjugated anti insulin	10μl	x1
E : HRP conjugated streptavidin	20μl	x1
F : Substrate chromogen reagent (TMB)	12 ml	x1
H: Reaction stopper(1M H2SO4)	12 ml	x1
I : Washing buffer concentrate (10x)	100 ml	x1

[**Preparation for assay Procedure**]

Prepare and use all regents after adaptation to the room temperature.

Standard Insulin solution(B) :

Standard Insulin solution dilution example.

・In the case of sample volume 10μl : Measurement range (10～0.156 ng/ml)

Concentration (ng/ml)	10	5.0	2.5	0.125	0.625	0.313	0.156	0
Std. Insulin solution (μl)→	10	100	100	100	100	100	100	0
Buffer solution (μl)	190	100	100	100	100	100	100	100

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Biotin conjugated anti insulin(D) : Dilute to 4,000 times by using buffer solution(C) and use.

HRP conjugated streptavidin(E) : Dilute to 2,000 times by using buffer solution(C) and use.

Washing buffer(I) : Dilute 10 times by using distilled water .

*Please use after melting right away.

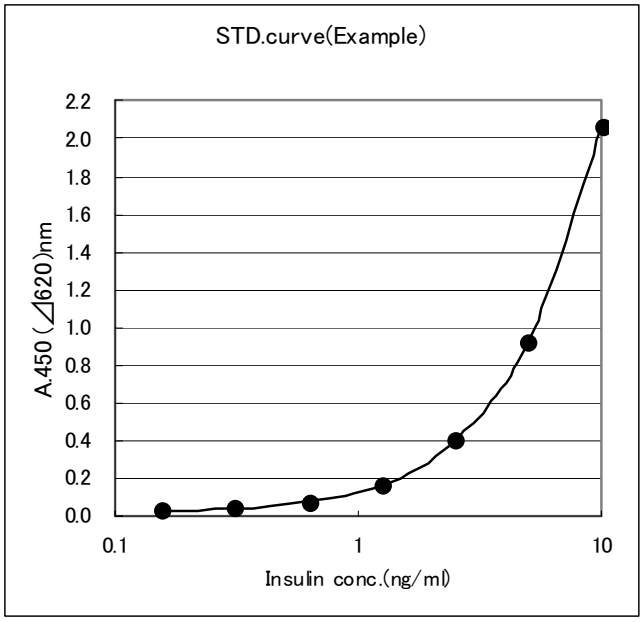
[**Assay Procedure**]

1) Rinse the anti insulin coated plate(A) 4 times with washing buffer(I).
2) Pipette 100μl of Biotin conjugated anti insulin(D) to each well.
3) Pipette 10μl sample or standard Insulin solution(B) into each well and shake.
4) Incubate for 2hours at room temperature. (20～25 ℃).
5) Rinse the plate 4 times with washing buffer(I).
6) Pipette 100μl of HRP conjugated streptavidin solution(E) into each well and shake.
7) Incubate for 30 minutes at room temperature .(20～25 ℃).
8) Rinse the plate 4 times with washing buffer(I).
9) Pipette100μl of Substrate chromogen reagent（F） into each well and shake.
10) Incubate for 30 minutes at room temperature. (20～25 ℃).
11) Pipette 100μl of Reaction stopper(H) into each well and shake.
12) Measure each well's absorbance at 450 nm (sub-wave length, 620nm) by the plate reader within 30 minutes.

[**Calculation of Insulin concentration**]

1. On logarithm paper, set the X-axis as the Insulin concentration(ng/ml),and the Y-axis as the absorbance.
2. Make the standard curve by plotting each absorbance of the serial dilution of standard insulin solution to the concentrations of each.
3. Plot the absorbance of sample on the standard curve ,and read the concentration of it.

＊Standard Curve Example.



[Preservation condition]

Store at 2 to 8℃

[Precautions]

1. Please read this manual carefully prior use.
2. This kit has been designed for research use only.
3. Do not use the reagents with different lot numbers together.
4. Do not spill the substrate-chromogen reagent and the acid stop solution on the skin or mucous membrane.

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Statements and Precautions as to Our Kits or their components (Please start working after reading these notes).

- This assay kit or its components should be used only for research works.
- The reagent solutions of the kit should be used principally immediately after dilution. Otherwise, keep them in a dark place at 2-8℃, and use them within 3 days.
- 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 are preserved for some period.
- Pipetting and dilution of the reagent solutions should be made accurately because these steps influence the assay precision.
- Do not dry the assay plate to avoid denaturation of the coated antibody or antigen.
- The reaction time should be counted from the onset of reagent pipetting.
- Prepare the standard curve in every assay. (For kits with standard solution.)
- Dilution of the assay sample must be carried out using the buffer solution attached to the kit.
- Preservation condition for the kit or its components should be strictly kept.
- Be careful not to allow the reagent solutions of the kit to contact with skin mucus and eyes (wearing glasses for protection is recommended). Especially treat the stopping solution very carefully because it contains sulfuric acid.
- HRP-conjugated reagent solution, chromogenic substrate solution, and reaction stopper should be avoided from contacting with any metal.
- In treating assay samples of animal origin, be careful for possible biohazards.