Advanced BioReagents

Insulin

For research use only

ABSbio™ Mouse/Rat Insulin ELISA Kit (SE010-insulin)

Insulin quantitative Assay

INTRODUCTION

ABSbio™ Mouse / Rat Insulin ELISA kit is used for the quantification of insulin in mouse and rat sera.

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to analyze the level of insulin in samples. The micro-plate is pre-coated with a monoclonal antibody against insulin. Standards and samples are added into the wells and co-incubated with a monoclonal antibody conjugated to horseradish peroxidase (HRP) enzyme. After wash step to remove any unbound substances, TMB substrate is added and color develops in proportion to the amount of insulin bound initially. The assay is stopped and the optical density of the wells determined using a micro-plate reader. Since the increases in absorbance are directly proportional to the amount of captured insulin, the unknown sample concentration can be interpolated from a reference curve included in each assay.

KIT COMPONENTS

Insulin Standards:	8 vials	Assay Diluent:	20 mL	TMB Solution:	12 mL	Plate sealer:	2
Detection Antibody (100x):	120 µL	Wash Solution (10x):	20 mL	Stop Solution:	6 mL	Anti-Insulin coated plate:	1

Storage and Handling: Shipping on ice. Store kit at 2-8 °C. Shelf Life: 6 months after receipt. Bring all reagents and materials to room temperature before assay. Remove any unused antibody coated strips from the micro-plate, return them to the foil pouch and re-seal.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips. 2. 96-well plate or manual strip washer. 3. Buffer and reagent reservoirs. 4. Paper towels or absorbent paper. 5. Plate reader capable of reading absorbency at 450 nm. 6. Distilled water or deionized water. 7. Micro-plate shaker capable of 300 rpm.

PREPARATION OF REAGENTS

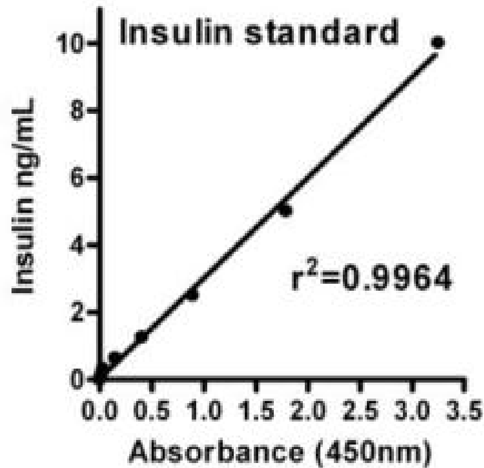
- Wash Solution: 10x dilute Wash Solution with dH_2O to prepare 1x Wash Solution.
- Detection Antibody: Prepare 1×Detection antibody solution by dilution of the 100×Detection antibody solution in Sample Diluent, mix well. 100 µl of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

PROCEDURE GUIDELINE

1. It is recommended that all standards and samples should be run in duplicate. Set standard wells, testing sample and blank wells on the assay plate/strip. Transfer diluted standard 5 µl to standard wells, diluted sample 5 µl to sample wells, sample diluent 5 µl only to blank wells.

	1	2	3	4	5	6	7	8	9	10	11	12
A	10ng/mL standard	10ng/mL standard	Undiluted sample 1	Undiluted sample 1	Undiluted sample 5	Undiluted sample 5						
B	5ng/mL	5ng/mL	3-fold dilute	3-fold dilute	3-fold dilute	3-fold dilute						
C	2ng/mL	2ng/mL	Undiluted sample 2	Undiluted sample 2	Undiluted sample 6	Undiluted sample 6						
D	1ng/mL	1ng/mL	3-fold dilute	3-fold dilute	3-fold dilute	3-fold dilute						
E	0.5ng/mL	0.5ng/mL	Undiluted sample 3	Undiluted sample 3	Undiluted sample 7	Undiluted sample 7						
F	0.1ng/mL	0.1ng/mL	3-fold dilute	3-fold dilute	3-fold dilute	3-fold dilute						
G	0.05ng/mL	0.05ng/mL	Undiluted sample 4	Undiluted sample 4	3- Undiluted sample 8	Undiluted sample 8						
H	blank	blank	3-fold dilute	3-fold dilute	3-fold dilute	3-fold dilute						

2. Add 100 µl of 1x Detection antibody solution per well. Cover the plate with plate sealer and incubate the plate at room temperature for 2 hrs or at 37 °C for 1h, shaking the plate at 300 rpm on a micro-plate shaker.
3. Decant as much liquid as possible, fill the wells with 200 µl wash solution, shaking for 1 min, decant the wash solution and remove residual liquid with absorbent paper. Repeat wash five times.
4. Add 100 µl of TMB Solution to each well and incubate at room temperature for 10~20 minutes protect from light, or keep close monitoring on the developing process until desired developing blue color observed. **Note: please be aware that the color may develop more quickly or more slowly that the recommended incubation time depending on the localized room temperature.**
5. Add 50 µl of Stop Solution to each well to stop the reaction (the blue color change to yellow), gently tap the plate frame for a few seconds to ensure thorough mixing.
6. Read absorbance of the plate on a microplate reader at 450 nm within 5 min.
7. Average the duplicate readings for each standard and samples, subtract the average zero (blank) standard optical density. Construct standard curve (plotting the mean OD450 for each standard on the X-axis against concentration on the Y-axis, draw a best-fit curve through the points) and calculate linear regression equation, then use corrected sample OD values and regression equation to calculate the corresponding sample concentration. It should be remembered that the sample has been diluted and its actual concentration should be justified by dilution factor (the measurement and calculation can be accomplished by software like SoftMax).



SUMMARY OF ASSAY PROCEDURE

Add 5 µl of standard or sample to each well.

Add 100 µl of 1×Detection antibody solution per well.

Incubate at room temperature for 2 hours (300 rpm).

Wash each well 5 times.

Add 100 µl of Substrate solution to each well.

Incubate at room temperature for 10-20 minutes.

Add 50 µl of Stop solution to each well.

Measure absorbance of each well at 450 nm.

Calculation

Typical Standard Curve

Always run your own standard curves for calculation of results.

ASSAY CHARACTERISTICS

A. Sensitivity
The lowest insulin level that can be measured by this assay is 0.05 ng/ml.

B. **Precision**
Intra-assay Precision (Precision within an assay) C.V. < 10%.
Inter-assay Precision (Precision between assays) C.V. <10%.

C. **Recovery**
The recovery of the assay was determined by adding various amounts insulin to a sample. The measured concentration of the spiked sample in the assay was compared to the expected concentration. The average recovery was 96-103 %.

D. **Specificity**
Percent of cross reactivity
Human insulin 100 %
Rat insulin 100 %

TROUBLE SHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure that all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with washing solution.