ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Wide Range Mouse Insulin ELISA Kit

(Catalog Number: 32100)

For the quantitative determination of insulin in mouse serum, plasma or fluid

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Version 2.0

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PRINCIPLE OF THE ASSAY

This assay is a two-site enzyme-linked immunosorbent assay (ELISA). The microtiter 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, 3,3′,5,5′-Tetramethylbenzidine (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 is determined using a microplate 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.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter Strips (96 wells), coated with a monoclonal antibody against insulin, sealed
2. 10×Wash buffer, 30 mL
3. Assay buffer,13 mL, ready for use
4. 100×Detection antibody solution, a monoclonal antibody against insulin conjugated to horseradish peroxidase, 0.12 mL
5. Insulin standard solutions, 0 ng/mL (5 mL), 1 ng/mL, 2.5 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL and 50 ng/mL (0.1 mL each), ready for use
6. Substrate solution, 12 mL, ready for use
7. Stop solution, 12 mL, ready for use
8. Plate cover

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. Horizontal micro-plate shaker capable of 600 rpm

STORAGE

The kit should be stored at 2-8°C upon receipt. Remove any unused antibody-coated strips from the microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Wash buffer

Prepare 1×Wash buffer by mixing the 10×Wash buffer (30 mL) with 270 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

B. 1× Detection antibody solution

Prepare 1×Detection antibody solution by dilution of the 100×Detection antibody solution in Assay buffer, 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.

SAMPLE HANDLING

No dilution of the sample is required in this assay. If a sample has an insulin level greater than the highest standard, the sample should be diluted with 0 ng/mL insulin standard solution and the assay should be repeated. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be run in duplicate.

1. Add 5 µL of standard or sample to its respective well.
2. Add 100 µL of 1xDetection antibody solution per well.
3. Seal the plate with a plate cover. Incubate at room temperature for 90 minutes, shaking the plate at 600 rpm on a horizontal micro-plate shaker.
4. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well. Incubate at room temperature for 20 seconds. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 4 washes.
5. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light**.
6. Add 100 µL of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
7. Measure absorbance of each well at 450 nm immediately.

CALCULATION

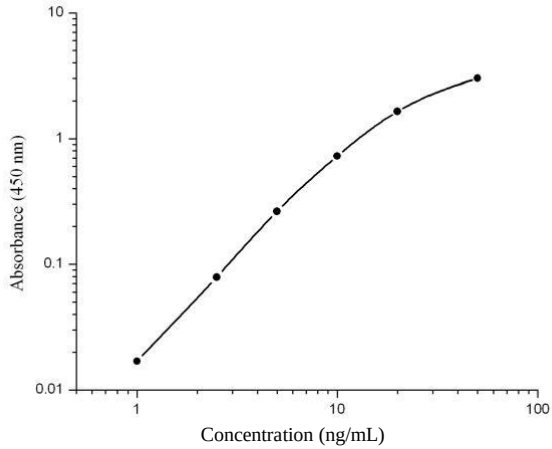
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against insulin concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Log-log curve fitting or curve of 4-parameter can be used for calculation.
3. Determine insulin concentration of samples from standard curve.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each assay.

Insulin (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.053	0
1	0.07	0.017
2.5	0.132	0.079
5	0.316	0.263
10	0.78	0.727
20	1.702	1.649
50	3.074	3.021

Insulin standard curve (log-log)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest insulin level that can be measured by this assay is 1 ng/mL.

B. Precision

Intra-assay Precision (Precision within an assay) C.V. <4.5%.
Inter-assay Precision (Precision between assays) C.V. <5.4%.

C. Recovery

The recovery of the assay was determined by adding various amounts of insulin to a sample. The measured concentration of the spiked sample in the assay was compared to the expected concentration. The average recovery was 91%.

D. Specificity

Percent of cross reactivity
Human insulin: 100%
Rat insulin: 100%

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 90 minutes (600 rpm).
↓
Wash each well 4 times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation