High sensitive mouse insulin immunoassay kit

Cat No: BIOK2000

Principal of the assay

This assay is a sandwich ELISA based on two monoclonal antibodies against separate antigenic determinants on the insulin molecule. The microplate is coated with a monoclonal antibody. Standards and samples are added into the wells and react with a monoclonal antibody conjugated to horseradish peroxidase (HRP) enzyme. After washing, TMB substrate is added to the wells and color develops in proportion to the amount of insulin bound. The assay is stopped and the intensity of the color is measured at 450 nm. The amount of insulin in the sample can be calculated from a standard curve.

Materials supplied

1	Microplate	96 wells
2	Wash buffer (10x)	20 ml
3	Assay buffer	9 ml
4	Detection antibody solution (100x)	0.09 ml
5	Insulin standard (0 ng/ml)	1 ml
6	Insulin standard (0.2 ng/ml)	0.1 ml
7	Insulin standard (0.5 ng/ml)	0.1 ml
8	Insulin standard (1.2 ng/ml)	0.1 ml

9	Insulin standard (3 ng/ml)	0.1 ml
10	Insulin standard (6.9 ng/ml)	0.1 ml
11	Substrate solution	12 ml
12	Stop solution	12 ml
13	Plate sealer	1

Other materials required

1. Pipettes and pipette tips.

2. Distilled water or deionized water.

3. Volumetric containers and pipettes for reagent preparation.

4. Paper towels or absorbent paper.

5. Multi-channel micropipettes or automated microplate washer.

6. Microplate shaker capable of 600 rpm.

7. Microplate reader capable of reading absorbance at 450 nm.

Storage

The kit should be stored at 2-8°C upon receipt. Remove any unused antibodycoated strips from the microplate, 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

A. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (20 ml) with 180 ml of distilled water or deionized water. 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. 70 µl of the 1×Detection antibody solution is required per well.

Preparation of samples

If a sample has a greater concentration of insulin than the highest standard, the sample should be diluted with 0 ng/ml insulin standard solution and the assay should be repeated.

Assay procedure

Note: All reagents and microplate strips should be equilibrated to room temperature prior to use. A standard curve must be performed with each assay. It is recommended that all standards and

samples should be run in duplicate.

1. Add 70 µl of 1x Detection antibody solution to each well.

2. Add 5 µl of each standard or sample to its respective well.

3. Cover the plate with a plate sealer. Incubate at room temperature for 2 hours, shaking the plate at 600 rpm on a microplate shaker. (*Alternative incubation step in the absence of shaker: gently tap the plate frame for a few seconds to ensure thorough mixing, incubate at room temperature for 3 hours.)

4. Discard well contents and remove any remaining solution by inverting and tapping the plate on a clean paper towel. Add 300 µl of 1× Wash buffer to each well. Incubate at room temperature for 30 seconds.

5. 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.

6. Add 100 µl of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**

7. Add100 µl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing. 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 curvefitting is recommended for data analysis.

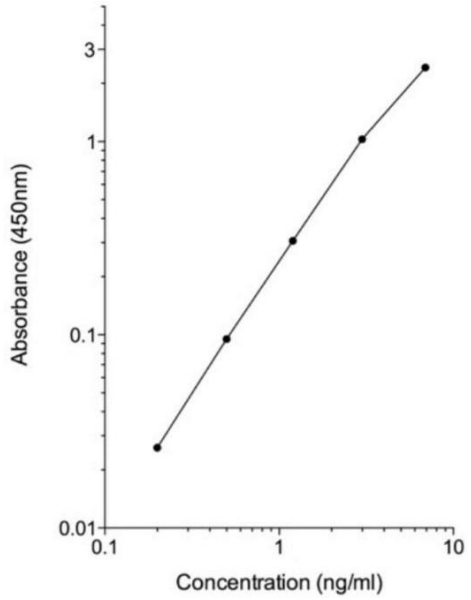
3. Determine insulin concentration of samples from standard curve.

Typical standard curve (600 rpm)

Note: The following standard curve is provided for demonstration only. Do not use it to determine actual assay results. A standard curve should be generated for each assay.

Insulin (ng/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.073	0
0.2	0.099	0.026
0.5	0.168	0.095
1.2	0.379	0.306
3.0	1.101	1.028
6.9	2.491	2.418

Insulin standard curve (log-log)



Assay characteristics

A. Precision

Intra-assay Precision (Precision within an assay) C.V <10%.

Inter-assay Precision (Precision between assays) C.V <10%.

B. Specificity

Percent of cross reactivity

Human insulin 100%

Rat insulin 100%

Summary of the assay procedure

Add 70 µl of 1× Detection antibody solution to each well.

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Add 5 µl of standard or sample to its respective well.

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Incubate at room temperature for 2 hour (600 rpm).

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Wash each well 4 times.

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Add 100 µl of Substrate solution to each well.

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Incubate at room temperature for 15 minutes.

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Add 100 µl of Stop solution to each well.

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Measure absorbance of each well at 450 nm.

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Calculation

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