ab285341 – Mouse Insulin ELISA Kit

For quantitative measurement of insulin in mouse serum, plasma, tissue homogenates, culture supernatants and other biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285341

Storage and Stability

The entire kit may be stored at 4°C for 6 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	1	4°C
Lyophilized Standard	2 vials	4°C
Sample and Standard dilution buffer	20 ml	4°C
Biotin- detection antibody (Lyophilized)	1 vial	4°C
Antibody dilution buffer	10 ml	4°C
HRP-Streptavidin Conjugate (SABC)	120 µl	4°C
SABC dilution buffer	10 ml	4°C
TMB substrate	10 ml	4°C
Stop Solution	10 ml	4°C
Wash buffer (25X)	30 ml	4°C
Plate sealers	5	4°C
Purified water	200 µl	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance of 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean Eppendorf tubes for preparing standards and sample dilutions
- Absorbent paper

Reagent Preparation

Δ Note: Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

Biotin- detection antibody working solution: Add 130 µl of Purified water to the lyophilized antibody and mix thoroughly. Store the solution at 4°C. Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.

HRP-Streptavidin Conjugate (SABC): Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.

Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, warm it with 40°C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Standard Preparation

1. Reconstitute the lyophilized Insulin standard by adding 1 ml of Standard/Sample Dilution Buffer to make the 5000 pg/ml standard stock solution. Use within 2 hours after reconstituting.
2. Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely.
3. The standard stock solution is Tube 1.
4. Add 0.3 ml of Standard/Sample Dilution Buffer to 7 additional tubes (Tubes 2-8).
5. Perform 2-fold serial dilutions by adding 0.3 ml of standard from Tube 1 to Tube 2, mixing well, then adding 0.3 ml from Tube 2 into Tube 3, mixing well, and so on to make the standard curve within the range of this assay.
   The last Tube (Tube 8) will be blank control (should only contain Standard/Sample Dilution Buffer).

   Δ Note: Suggested standard concentrations (Tube 1 – Tube 8) are: 5000, 2500, 1250, 625, 312.5, 156.25, 78.1, 0 pg/ml

Sample Preparation

Δ Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

Serum: Coagulate the serum for 2 hours at room temperature or overnight at 4°C. Centrifuge at approximately 1000×g for 20 min. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15 min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000xg. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

Tissue homogenates: Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess hemolysis blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9 mL PBS would be appropriate for 1 g of tissue. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to retrieve the supernatant.

Cell culture supernatant: Centrifuge supernatant for 20 minutes to remove insoluble impurity an d cell debris at 1000×g at 4°C. Collect the clear supernatant and carry out the assay immediately or aliquot and store at -20°C.

Cell culture lysate: Use commercial RIPA kits to follow the instructions provided. For 2 x 106 cells, use 0.5 ml RIPA lysis buffer. The total protein concentration should be determined by BCA kit and must not exceed 0.3 mg per sample.

Other biological fluids: Centrifuge samples for 20 min at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.

Δ Note: End user should estimate the concentration of the target protein in the test sample first and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

Assay Protocol

Δ Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed
2. Wash plate 2 times with 1X Wash Buffer before adding standard, sample and control wells.
3. Dilute samples with Sample/Standard dilution buffer in at least a 1:2 ratio.
4. Add 100 µl of each prepared Standard or Sample into the appropriate wells. Cover well and incubate for 1.5 hours at 37°C.
5. Remove the cover and discard the plate content and wash 2 times with 1X Wash Buffer. Do not allow the wells to dry completely at any time.
6. Add 0.1 ml of Biotin-detection antibody work solution into the above wells. Seal the plate and incubate at 37°C for 60 min.
7. Discard the solution and wash 3 times with 1X Wash Buffer. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or auto washer. Let it soak for 1-2 minutes, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
8. Add 0.1 ml of SABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
9. Discard the solution and wash 5 times with 1X Wash Buffer as step 7.
10. Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37 °C in the dark within 10-20 mins. The shades of blue should be seen in the first 3-4 wells by the end of incubation.
11. Add 50 µl of Stop Solution to each well.

Measurement

Read absorbance at 450 nm within 20 minutes of adding stop solution. Subtract the absorbance of the blank from the readings for each standard and sample.

Calculation

- For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well).
- The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Mouse Hepcidin concentration of the samples can be interpolated from the standard curve.
- If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)