Human Insulin Degrading Enzyme (IDE) ELISA Kit – Biotin Detection Antibody Format 96T

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FIVEphoton Biochemicals

For research use only.
Not for diagnostics.

Part No. hIDE-Biotin(96T)

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Store: 2-8^{o}C. Expiration: 6 months after arrival.
Safety: Stop solution contains acid. Avoid contact and inhalation. Wear eye protection.

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Biotin Detection Antibody Format

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Storage: 2-8^{o}C, expires six months after arrival

Safety: Stop solution contains acid. Avoid eye and skin contact

Standard Peptide: 192 ng/ml

Assay Range: 600 pg/ml - 180 ng/ml

Sensitivity: 544 pg/ml

Overview: Please familiarize yourself with this protocol prior to performing the assay. It is recommended that the researcher overviews the literature to identify optimal and detailed methods for sample preparation; the sample isolation methods discussed in this protocol serve only as a general guide. Blanks, Standards and Samples should be measured at least in duplicate to account for pipetting error.

The kit measures Human Insulin Degrading Enzyme in sandwich ELISA format. The ELISA plate is provided pre-coated with an anti-Insulin Degrading Enzyme monoclonal antibody. The experimenter mixes the samples with a provided polyclonal anti-Insulin Degrading Enzyme antibody labeled with biotin and with the provided streptavidin-HRP solution. This sample-antibody-biotin-streptavidin-HRP mixture is then added to the ELISA plate, which is incubated for 60 min at 37^{o}C. Unbound biotin-linked antibody is washed away and color reagents are added that change the color of the solution to blue. The blue solution then turns yellow after addition of the stop solution. The absorbance of the resulting yellow solution at 450nm corresponds to the concentration of Human Insulin Degrading Enzyme (IDE) in the samples.

Sample Preparation:

1. Note that samples containing NaN_3 may inhibit Horse Radish Peroxidase (HRP) and the color reaction.
2. If possible, assay the sample immediately after isolation. Otherwise store samples at -20^{o}C without freeze-thawing cycles.
3. **Serum**: Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant carefully. If particulates are detected after storage, centrifuge again and collect the supernatant for assays. A significant dilution of serum in the sample diluent solution (for example 200-1000X) may be required to maintain specificity of the assay.
4. **Blood plasma**: In accordance with the requirements of sample collection, EDTA or sodium citrate should be used as the anti-coagulant. Add EDTA or sodium citrate and mix 10-20 minutes. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatants carefully. If particulates are detected after storage, centrifuge again

and collect the supernatant for assays. A significant dilution of plasma in the sample diluent solution (for example 200-1000X) may be required to maintain specificity of the assay.

5. **Urine**: Collect by sterile tube. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatants carefully. When particulates appear due to storage, centrifuge again and collect the supernatant to assay.

6. **Cell culture supernatant**: Collect in sterile tubes when examining secreted components. Centrifuge at 2000-3000 RPM for approximately 20 minutes to sediment cells. Collect the supernatants carefully.

7. **Intracellular and membrane bound components**. To assay intracellular components, use a non-denaturing cell lysis buffer to lyze membranes and solubilize cytoplasmic and membrane bound components. Add ice cold lysis buffer with protease inhibitors to cells. Keep the tube in ice for 30 min. Tap the tube several times during this period to mechanically shear membranes. Centrifuge cellular debris in a microcentrifuge at 18000 rpm for 15 min. Collect the supernatant to assay.

8. **Tissue sample**: Use a homogenization protocol in a non-denaturing buffer to release protein components. Add protease inhibitors and keep the solution ice cold during the homogenization steps. Centrifuge debris and assay the supernatant.

Do not use denaturing cell lysis buffers such as RIPA for protein extraction.

9. Samples can be aliquoted and stored at -80^{o}C for later use.

10. Samples can be diluted in a sample dilution solution with the formula: 1% BSA in PBS pH 7.4. A 1:5 dilution is recommended. i.e. 10 μl sample, 40 μl sample dilution solution

Table 1. Materials supplied. Store all materials at 4^{o}C

1	Standard peptide: 192 ng/ml	0.5ml	7	Chromogen Solution A	6ml
2	Standard diluents (1% BSA in PBS pH 7.4	3 ml	8	Chromogen Solution B	6ml
3	Anti-Insulin Degrading Enzyme Coated ELISA plate (96T)	12 well× 8 strips	9	Stop Solution	6ml
4	Streptavidin-HRP	6 ml	10	Instruction Manual	1
5	20×wash solution	20 ml	11	Closure Membrane	2
6	Anti Insulin Degrading Enzyme antibody labeled with biotin (detection antibody)	1ml	12	Sealed bags	1

Materials required but not supplied

1. Sample dilution buffer (1% BSA in PBS pH 7.4)
2. 37^{o}C incubator
3. Standard absorbance plate reader
4. Precision pipettes and disposable pipette tips
5. De-ionized water
6. Disposable tubes for sample dilution
7. Absorbent paper

Important notes and preparation for the assay

1. Make a solution that is 1% BSA in PBS pH 7.4 for dilution of sample (i.e. "sample dilution solution").
2. Equilibrate the ELISA plate strips to room temperature for at least 30 minutes. The plate strips should be kept in a zip lock bag to prevent drying of the wells. Store unused sections of the ELISA plate in a sealed bag at 4°C.
3. Use new pipettes tips at each step to prevent contamination.
4. Do not use reagents from other ELISA kits..
5. Substrate B is light sensitive. Limit light exposure.
6. **Manual Wash Method:** Gently aspirate off the liquid in each well. Turn over the plate and pat-dry on absorbent paper. Add 100µl wash solution and repeat 5 times. Therefore 600ul wash solution is needed per well. An automatic washer can also be employed to wash the ELISA wells.

Assay procedures

Blanks, standard and sample preparation: Prepare the blanks, standards and samples in a separate 96-well dish and transfer simultaneously to the ELISA plate. Do not prepare samples in the ELISA plate.

Assay procedure

1. Standard dilutions. Use Table 2 as a guide for dilutions of standards in the standard diluent solution. Make dilutions in separate tubes or multi-well dishes, not in the ELISA dish.

Table 2. Standard Dilutions

Standard Concentration	Standard Number	Dilution Instructions
96 ng/ml	5	120 l original standard + 120 l standard diluents solution, mix
48 ng/ml	4	120 l standard No.5 + 120 l standard diluents solution, mix
24 ng/ml	3	120 l standard No.4 + 120 l standard diluents solution, mix
12 ng/ml	2	120 l standard No.3 + 120 l standard diluents solution, mix
6 ng/ml	1	120 l standard No.2 + 120 l standard diluents solution, mix

2. Blank, standard and sample preparation: (Pre-mix solutions in a separate multiwell dish and transfer the solutions simultaneously to the ELISA dish. Do not pre-mix solutions in the ELISA dish).
   a) Blank wells: Only add chromogen solutions A and B and then stop solution during the procedural steps.
   b) Standard solution wells: Add 50 l standard and 50 l streptavidin-HRP (detection antibody labeled with biotin has been added in advance to the standards, therefore detection antibody is not added).
   c) Sample well: Dilute 10ul sample in 30 l sample diluent solution (1% BSA in PBS pH 7.4 – made by the experimenter), then add 10 l Insulin Degrading Enzyme detection-biotin antibody and 50 l streptavidin-HRP. Mix gently; do not vortex. Transfer the solutions to the ELISA dish. Seal the wells with a ziplock bag and rotate the plate gently for 60 minutes at 37°C.

3. Preparation of wash solution: Dilute the 30X wash solution to 1X with dH_20. Prepare 600μl 1X wash solution per well.
4. Wash: Carefully remove the seal plate membrane or zip lock bag that protects the wells from drying as not to cross-contaminate liquid. Aspirate off the liquid. Fill each well with 100ul wash solution. Aspirate the liquid after 30 seconds. Then repeat the wash procedure five times. Blot dry the plate.
5. Color development: First add 50 l chromogen solution A to each well and then add 50 l chromogen solution B to each well. Shake gently to mix solutions A and B. Incubate protected from light for 10 minutes at 37°C.
6. Stop: Add 50 l Stop Solution to each well to stop the reaction (the blue color changes into yellow).
7. Read samples at 450nm within 10 min of adding the stop solution: Set the blank well as zero, measure the absorbance (OD) of each well at 450nm.

Data Analysis

1. Compile a standard curve using the blank standard solutions and the corresponding OD values. You may wish to calculate a linear regression equation from the standard curve to determine the concentration of your samples. Take into account dilution factors in your calculation of Insulin Degrading Enzyme concentration. Alternative methods of data analysis to calculate concentrations of your samples can also be employed.

Flow chart of the procedures

Separately prepare standards, blank and samples with detection antibody and HRP-streptavidin as required

Incubate with the ELISA plate for 1 hr at 37°C.

Wash the plate five times.

Add Chromogen solutions A and B. Incubate for 10 min at 37°C for color development.

Add stop solution

Measure OD value at 450nm within 10 min