Insulin Degrading Enzyme (IDE) ELISA Kit

General Protocol

FIVEphoton Biochemicals

For research use only.
Not for diagnostics.

Part No. IDE-ELISA

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This protocol is for reference only. Use the protocol included with the kit for your experiments.

Store: 4°C. Expiration: 6 months after arrival.
Safety: Stop solution contains acid. Avoid all contact and inhalation. Wear eye protection.

Insulin Degrading Enzyme ELISA Kit

Part No. IDE-ELISA

(Human; hIDE-ELISA. Mouse; mIDE-ELISA. Rat; rIDE-ELISA. Canine; cIDE-ELISA

For research only. Not for diagnostic applications.

Storage: 4°C, six months after arrival

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

Standard Peptide: 22.5 – 72.0 pg/ml depending on kit.

Assay Range: 0.7 - 54 pg/ml depending on kit.

Experimental Principles

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to measure Insulin Degrading Enzyme concentration. Samples are applied in microelisa wells pre-coated with an affinity purified polyclonal anti-Insulin Degrading Enzyme antibody. Samples are incubated and then washed. A second goat anti- Insulin Degrading Enzyme-HRP conjugate antibody is added, followed by incubation and wash. Chromogen solutions A and B are added resulting in a coloration change to blue. A stop solution is applied to terminate the reaction, turning the solution to yellow. Absorbance readings at 450 nm corresponding to standard peptide concentrations is used to correlate the concentration of Insulin Degrading Enzyme in the samples.

Insulin Degrading Enzyme has been detected in the cytosol, peroxisomes, cell surface and extracellular medium[1,2]. Therefore, there are several protein isolation methods to extract this enzyme for ELISA assay depending on what cellular pool the researcher has interest. This ELISA kit detects Insulin Degrading Enzyme in a variety of sources, including serum, CSF, plasma, cell culture supernatant, tissue homogenates and cell lysates. Prepare the samples as described below.

Reference

1. Konstantinos Vekrellis, et al. 2000. The Journal of Neuroscience 20(5):1657–1665.
2. Ji Zhao, et. al. 2009. Molecular Neurodegeneration 2009, **4**:4 doi:10.1186/1750-1326-4-4

Sample Preparation: Use 50 ml tubes and a desktop cell culture centrifuge for the following procedures.

1. **Serum**: Coagulate at room temperature for 10-20 min. Centrifuge for 20 min at 2000-3000 rpm. Remove supernatant, If precipitation appears, centrifuge again. Assay the supernatant fraction.
2. **Plasma:** Use suitable EDTA or heparin as an anticoagulant. Mix for 10-20 min using a stir bar. Centrifuge for 20 min at 2000-3000 rpm. Remove supernatant, If precipitation appears, centrifuge again.
3. **Urine**: Collect in a sterile container. Centrifuge 20-min at 2000-3000 rpm. Remove supernatant, If precipitation appears, centrifuge again. Remove supernatant.
4. **Cell culture supernatant:**. Detection of secretory components: Centrifuge culture media for 20 min at 2000-3000

rpm. Remove supernatant.

5. **Cell cytoplasm:** Dilute cell suspension with PBS (pH7.2-7.4) to a cell concentration of 1 million cells / ml. Perform repeated freeze-thaw cycles to fracture the cell membrane and to release intracellular components. Centrifuge for 20min at 2000-3000 rpm. Remove supernatant, If precipitation appears, centrifuge again.
5. **Tissue:** Cut and weigh tissue slice. Add slice in PBS (pH7.2-7.4). Freeze rapidly with liquid nitrogen. Thaw sample to 2-8^{o}C, add PBS and homogenize. Centrifuge for 20 min at 2000-3000rpm. Remove supernatant.
6. Samples can be store at -80^{o}C. Avoid repeated freeze-thaw cycles. You may aliquot samples for later ELISA assays.
7. **Avoid denaturing cell lysis buffers that contain SDS such as RIPA buffer.**

Table 1. Materials Included with Kit. Store all materials at 4^{o}C

1	Standard peptide	0.5ml		7	Chromogen Solution A	6ml
2	Standard diluent	1.5 ml		8	Chromogen Solution B	6ml
3	Microelisa Strip plate	12 well×8strips		9	Stop Solution	6ml
4	HRP-Conjugate Antibody	6 ml		10	Instruction Manual	1
5	30×wash solution	20ml		11	Sealed bags	1
6	Sample Diluent	6ml				

Materials required but not supplied

1. 37^{o}C incubator
2. Standard absorbance microplate reader
3. Precision pipettes and disposable pipette tips
4. De-ionized water
5. Disposable tubes for sample dilution
6. Absorbent paper
7. 96 well dishes for preparation of solutions prior to transfer to the ELISA dish
8. 96-channel transfer pipette

Important notes and preparation for the assay

1. The experimenter should perform preliminary tests to identify the sample dilutions that meet the assay range. Perform a preliminary assay with your samples, using the standard peptide at the low and high concentrations of the assay range of this kit. Suspend and dilute experimental samples into the “Sample Diluent (Table 1, Component 6)” to meet the assay range, (alternatively, dilute samples in PBS). A dilution series of several samples may be required to identify the correct sample concentration that meets the assay range. Concentrate or dilute experimental samples if adjustment is needed. Set aside sufficient experimental samples for reserve to repeat the assay.
2. Determine whether the vehicle buffer inadvertently cross-reacts with the assay and generates a color change by performing vehicle only controls. Additionally, determine whether ingredients in the vehicle buffer inhibit the assay reaction by diluting the provided standard peptide in the vehicle and perform an assay test. Compare results to the same standard peptide dilution in the Sample Diluent (Table 1, Component 6). For remedy, dilute samples in “Sample Diluent” (Table 1, Component 6) or prepare samples in another vehicle (such as PBS) to prevent

inadvertent experimental readings or assay inactivation.

3. The kit should be equilibrated to room temperate for 30 min prior to performing the assay. Store opened microelisa plates in a sealed plastic bag at 4°C. A multi-channel pipettor is the recommended method to simultaneously apply samples. Plates should be sealed during the assay. Wells should not be allowed to dry.
4. Perform preparations of standards and samples in separate tubes or 96-well plates, not in the ELISA plate wells. Transfer standards and samples simultaneously to the ELISA plate.
5. It is recommended that samples are assayed in duplicate to address pipetting error.
6. Use new applicator tips and ELISA plate sealants to avoid cross-contamination.
7. Do not mix reagents from other ELISA kits.
8. Note that sodium azide in samples that is not washed away may inhibit horse-radish peroxidase (HRP) that generates the color reaction of the assay.
9. When calculating concentration of your sample from the assay, make sure to take into account the dilution factor.
10. If the wash solution crystallizes during storage at 4°C, heat the solution at 37°C and shake until crystals suspend.

Assay procedures

Standard and Sample Preparation. Standards and Samples should be added simultaneously to wells**. Prepare the standards and samples in a separate 96-well dish and transfer simultaneously to the ELISA dish.**

Assay procedure

1. Set aside and mark 14 wells for standard peptide dilutions. Configure seven concentrations of standard peptide in duplicate as indicated in Table 2 below. Do not use the ELISA wells directly to perform the dilutions. The final total volume in each well should be 50µl.

Table 2. Standard Dilutions (This dilution series is for reference only with a 450 pg/ml standard. The dilution series will depend on the standard peptide concentration and is detailed in the protocol shipped with the kit).

Well	Standard Concentration	Standard Number	Dilution Instructions
1	400 pg/ml	1	Mix 100µl Standard Peptide (Table 1, Component 1) with 50µl Standard Diluent (Table 1, Component 2). Remove 100µl to make standard 3.
2	400 pg/ml	2	Mix 100µl Standard Peptide with 50µl Standard Diluent. Remove 100µl to make standard 4.
3	267 pg/ml	3	Mix 100µl Standard Number 1 with 50µl Standard Diluent. Remove 100µl to make standard 5.
4	267 pg/ml	4	Mix 100µl Standard Number 2 with 50µl Standard Diluent. Remove 100µl to make standard 6.
5	133 pg/ml	5	Mix 100µl Standard Number 3 with100µl Standard Diluent. Remove 100µl to make standard 7. Remove 50µl, discard.
6	133 pg/ml	6	Mix 100µl Standard Number 4 with100µl Standard Diluent. Remove 100µl to make standard 8. Remove 50µl, discard.
7	67 pg/ml	7	Mix 100µl Standard Number 5 with 100µl Standard Diluent. Remove 100µl to make standard 9. Remove 50µl, discard.
8	67 pg/ml	8	Mix 100µl Standard Number 6 with 100µl Standard Diluent. Remove 100µl to make standard 10. Remove 50µl, discard.
9	33 pg/ml	9	Mix 100µl Standard Number 7 with 100µl Standard Diluent. Remove 50µl to make standard 11. Remove 100µl, discard.
10	33 pg/ml	10	Mix 100µl Standard Number 8 with 100µl Standard Diluent. Remove 50µl to make standard 12. Remove 100µl, discard.
11	16 pg/ml	11	Mix 50µl Standard Number 9 with 50µl Standard Diluent. Remove 50µl to make Standard

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			13.
12	16 pg/ml	12	Mix 50µl Standard Number 10 with 50µl Standard Diluent. Remove 50µl to make Standard 14.
13	8 pg/ml	13	Mix 50µl Standard 11 with 50µl Standard Diluent. Disgard 50µl.
14	8 pg/ml	14	Mix 50µl Standard 12 with 50µl Standard Diluent. Disgard 50µl.

2. Set up 2 blank wells separately. In blank wells, add 40µl of the provided Sample Diluent (Component 6) and 10µl vehicle buffer that the sample is in; do not add sample. Perform all other procedures of the assay, except omit HRP-conjugate antibody (Table 1, Component 4).

3. For wells with experimental samples, add 40µl provided Sample Diluent for each well, then add 10µl of experimental sample. This creates a 5X dilution factor of sample which should be accounted for later when calculating sample concentration. This preparation should be done in separate tubes or 96-well plates, and not in the ELISA dish.

4. Transfer the standard peptide solutions, blank well solutions and diluted experimental samples simultaneously to the ELISA dish. Use the closure membrane to enclose the plate, mix gently with a rotator table, and incubate for 30 min at 37°C, or 1 hr at RT.

5. Dilute the 30X Wash Solution (Table 1, Component 5) with dH_20. Make 3 ml of diluted wash solution for each assay well.

6. After the first incubation period, discard the liquid in the wells by gently aspirating. Turn the plate upside down and gently pat dry the plate with absorbent paper. To wash the wells, simultaneously fill each well with 100 µl of diluted Wash Solution, oscillate gently with the rotator table for 30 sec, and then aspirate off liquid. Pat dry the ELISA microplate with absorbent paper. Repeat the wash steps 5 times.

7. Prepare in a separate 96-well dish 50 µl HRP-conjugate reagent (Table 1, Component 4) for each well, except the blank wells. Simultaneously transfer 50 µl HRP-conjugate reagent to each well, except for the blank wells. Rotate gently for 1 hr at 37°C for 30 min or 1 hr at RT.

8. Wash the wells 5X as described above in step 6. Remove liquid from the wells. Do not allow wells to dry.

9. In a separate 96-well plate, mix 50 µl Chromogen Solution A (Table 1, Component 7) with 50µl of Chromogen Solution B (Table 1, Component 8) for each well. Transfer the chromogen mixture to each well. Mix the ELISA plate gently for 15 min at 37°C in the dark.

10. Simultaneously add 50 µl of Stop Solution (Table 1, Component 9) to each well. Upon addition of stop solution, the blue color should immediately change to yellow.

11. Measure the optical density (OD) at 450 nm within 15 minutes after adding stop solution. Set the blank wells as zero.

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Data Analysis

1. Compile a standard curve using the blank standard solutions and corresponding OD values. You may wish to calculate a linear regression equation to determine the concentration of your samples. Remember that samples were diluted 5 fold in the assay in your final calculation. Other data analysis methods to calculate sample concentrations are also applicable.

Flow chart of the procedures

Prepare standards, blank and samples

Add samples to wells, incubate for 1 hr at RT or 30 min at 37°C.

Wash each well five times.

Add HRP-conjugate antibody to each well, incubate for 1 hr at RT or 30 min at 37°C.

Wash each well five times

Add chromogen solutions A and B, 15 min at 37°C, dark

Add stop solution

Measure OD 450 nm within 15 min

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