Human Presenilin 1 ELISA Kit

Part hPS1-ELISA

FIVEphoton Biochemicals

For research use only.
Not for diagnostics.

Part No. hPS1- ELISA

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

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Human Presenilin 1 ELISA Kit

Part No. hPSI-ELISA
For research only. Not for diagnostic applications.

Storage:
2-8°C, expires six months after arrival

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

Standard Peptide Concentration: 900 pg/ml
Assay Range: 10-700 pg/ml
Sensitivity: 10 pg/ml
For research use only. Not for diagnostic applications.

Overview: Please familiarize yourself with this protocol prior to performing the assay. It is recommended that the researcher overviews the literature to identify 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. Do not smudge or scratch the bottom or sides of the ELISA dish. Wipe off all condensation on the plate exterior.

Overview:
The kit measures Human Presenilin 1 in sandwich ELISA format. The ELISA plate is provided pre-coated with an affinity purified anti-Presenilin antibody. The experimenter applies experimental samples and then adds the provided anti-PS-1 biotin labeled detection antibody and streptavidin-HRP. This sample-antibody-biotin-streptavidin-HRP mixture is incubated on the ELISA plate for 60 min at 37°C. Unbound HRP-biotin-linked antibody complex is afterwards washed away, and color reagents are added that change the color of the solution to blue. The blue solution turns yellow after stop solution is added. The absorbance at 450 nm of the resulting yellow solution corresponds to the concentration of Human Presenilin 1 in the samples.

Sample Preparation: The discussion below is provided only as a general guide. Prior to performing the assay, the researcher should identify sample preparation methods in detail that are relevant toward their experiments.

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 -80°C without

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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 (for example, 200-1000X fold) of serum in a sample diluent solution may be required to maintain assay specificity.

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 (for example, 200-1000X fold) of plasma in a sample diluent solution may be required to maintain assay specificity.

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 with protease inhibitors to solubilize membranes and suspend cytoplasmic and membrane bound proteins. Keep the tube with the lysate on ice for 30 min during the lysis step. Tap the tube several times during this period to further mechanically shear membranes. Centrifuge cellular debris in a microcentrifuge at 18000 rpm for 15 min. Collect the supernatant that will be used in the 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°C for later use.

Table 1. Materials supplied. Store all materials at 4°C. If particulates are observed in any of the reagents, centrifuge and use the clarified supernatant.

1	Standard	0.5ml	8	Chromogen Solution A	6ml
2	Standard diluent	3 ml	9	Chromogen Solution B	6ml
3	Antibody coated ELISA plate (96T)	12 well× 8 strips	10	Stop Solution	6ml
4	Biotin labeled anti-PS-1 Detection Ab	1 ml			
5	HRP -Streptavidin- Solution	6 ml	11	Instruction Manual	1
6	30× wash solution	20 ml	12	Closure Membrane	2
7	Sample Diluent	6 ml	13	Sealed bags	1

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Materials required but not supplied

1. 37°C incubator
2. Rocker table to gently mix ELISA dish inside 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. Equilibrate the ELISA plate strips and other reagents used in the assay to room temperature for at least 30 minutes. The plate strips should be stored 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.
2. Use new pipettes tips at each step to prevent contamination.
3. Do not use reagents from other ELISA kits.
4. Do not smudge or scratch well bottom or sides. Make sure plate exterior is dry and bubbles in the wells are not present.
5. Substrate B is light sensitive. Limit light exposure.
6. **Manual Wash Method:** Prepare 600ul 1X wash solution from the provided 30X wash solution for each well. After the 60 min incubation period, gently aspirate off the liquid in each well. Turn over the plate and pat-dry on absorbent paper. Add 100ul wash solution and let percolate in the wells for 3 minutes prior to aspirating. Repeat the wash steps five times for 30 second each wash. An automatic washer can also be employed to wash the ELISA wells.
7. **Assay Range Dilution of Samples: Very Important**: Perform preliminary tests to identify the appropriate dilution of your samples to match the linear range of the ELISA kit. For example, remove a 8-well strip, prepare one well with the provided standard peptide at the lowest concentration and another well with standard peptide at the highest concentration of the assay range. Set another well as the bank well (see blank preparation below). Perform a dilution series of your representative samples with five diluted samples at 10X diluted increments. Then perform the assay using the protocol described below. Identify the dilution factor that provides an average measurement at approximately mid-point of the assay range. Dilute your samples with the provided sample diluent at this ratio prior to applying the samples to the ELISA plate. At completion of the assay, multiply by the dilution factor to calculate the sample concentrations.

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. Wear gloves and eye protection.

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Assay procedure

1. Standard dilutions. Use Table 2 as a guide for dilutions of standards in the "standard diluent" provided with the kit (Table 1). Make dilutions in separate tubes or a separate multi-well dish, but NOT in the ELISA dish.

Table 2. Standard Dilutions

Standard Concentration	Standard Number (tube #)	Dilution Instructions
600 pg/ml	5	Mix 120µl standard with 60µl standard diluent. Remove 120µl and dispense in tube #4.
400 pg/ml	4	Add 60µl standard diluent and mix. Then remove 60µl and add to tube #3
200 pg/ml	3	Add 60µl standard diluent and mix. Then remove 60µl and add to tube #2
100 pg/ml	2	Add 60µl standard diluent and mix. Then remove 60µl and add to tube #1
50 pg/ml	1	Add 60µl standard diluent and 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: The blank solution reproduces the content of the sample solution in sample diluent without the antigen. Set up two "blank wells:" Reproduce the ratio of sample vehicle (*without* sample) relative to the Sample Diluent . For each blank well, prepare 40 µl of mixed "blank solution."

b) Standard solution wells: Dispense 40µl of standard at each dilution.

c) Sample wells: For each well, prepare 40µl sample (that may have been previously diluted in Sample Diluent to meet the assay range).

d) Simultaneously dispense blanks, standards and samples into the ELISA strip.

e) Dispense 10µl biotin-labeled detection antibody and then 50µl Steptavidin- HRP to each well. Cover the plate with the closure membrane.

f) Mix gently in a 37°C incubator for 60 min.

3. During the incubation period, prepare the wash solution: Dilute the 30X wash solution to 1X with dH_20. Prepare 600ul 1X wash solution per well.

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4. Wash: Carefully remove the closure membrane: do not cross-contaminate liquid. Gently aspirate off the liquid in each well. Turn over the plate and pat-dry on absorbent paper. Add 100ul wash solution and let percolate in the wells for 3 minutes prior to aspirating. Gently rotate the ELISA plate during the 3 min period. Repeat the wash steps 5 times with 30 second washes. An automatic washer can also be employed to wash the ELISA wells. Blot dry the plate, but do not allow the wells to dry.

5. Color development: First simultaneously add 50µl chromogen solution A to each well and then simultaneously add 50µl chromogen solution B (which is light sensitive) to each well. Gently mix solutions A and B. Incubate protected from light for 10 minutes at 37°C.

6. Stop: Add 50ul Stop Solution to each well to stop the reaction (the blue color changes into yellow). Wear eye protection: the stop solution contains acid.

7. Read samples at 450nm within 10 min of adding the stop solution: Set the blank wells 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. Alternative methods of data analysis to calculate concentrations of your samples can also be employed.

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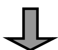
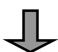
Flow chart of the procedures

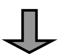
Separately prepare standards, blank and samples with detection antibody as required



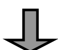
Incubate with the ELISA plate for 60 min at 37°C.



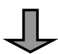
Wash the plate`



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

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