Human BACE1 ELISA Kit

Part No. hBACE1-ELISA

FIVEphoton Biochemicals

For research use only. Not for diagnostics or therapeutics.

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

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Part No. hBACE1 -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 Concentration: 225pg/ml

Assay Range: 5 - 200pg/ml

Sensitivity : 2.5pg/ml

Experimental Principles

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to measure BACE1 concentration. Samples are applied in microelisa wells pre-coated with an affinity purified polyclonal anti- BACE1 antibody. A second goat anti- BACE1-HRP conjugate antibody is added, followed by a 30 min incubation at 37°C. Unbound sample and detection antibody are washed, and then 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 450nm corresponding to standard peptide concentrations are used to determine the concentration of BACE1 in the samples.

As BACE1 is a transmembrane protein processed in the secretory pathway, including the endoplasmic reticulum, Golgi and plasma membrane [1], its primary method for extraction from cells and detection in ELISA assays involves preparation by cell lysis and solubilization using a non-denaturing detergent buffer. BACE1 has also been detected in biological fluids such as CSF [2]: Prepare the samples as described below depending on which fraction you plan to assay. Note that Instruction 7 is relevant for the membrane embedded fraction.

References:

1. Yan et. al. J Biol Chem. 2001 Sep 28;276(39):36788-96. Epub 2001 Jul 20.
2. Zetterberg H et al. Arch Neurol. 2008 Aug;65(8):1102-7.

Sample Preparation: The following serves as a generic guide for sample preparation. The research should perform an in-dept literature analysis to determine the optimal method to prepare samples.

1. **Serum**: Coagulate at room temperature for 10-20 min. Centrifuge for 20 min at 2000-3000 rpm. Collect supernatant for assay. 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. Collect supernatant. If precipitation appears, centrifuge again.
3. **Urine**: Collect in a sterile container. Centrifuge 20-min at 2000-3000 rpm. Collect supernatant, If precipitation appears, centrifuge again. Collect supernatant for assay.
4. **Cell culture supernatant:**. Detection of secretory components: Centrifuge culture media for 20 min at 2000-3000 rpm. Collect supernatant for assay.
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. Collect supernatant for assay. If precipitation appears, centrifuge again and assay supernatant.

6. **Tissue (Cytoplasmic components):** Cut and weigh tissue slice. Add slice in PBS (pH7.2-7.4). Freeze rapidly with liquid nitrogen. Thaw sample to 2-8°C, add PBS and homogenize. Centrifuge for 20 min at 2000-3000rpm. Remove supernatant.
7. **Cell and Tissue Homogenates and Lysates:** Use a non-denaturing detergent protein extraction reagent (for example, FIVEphoton Biochemicals Part No. ELSP-1) to homogenize tissues/and lyse cells in the presence of protease inhibitors on ice. Centrifuge the cellular debris and use the supernatant for the ELISA assay.
8. Samples can be store at -80°C. Avoid repeated freeze-thaw cycles. You may aliquot samples for later ELISA assays.
9. **Avoid denaturing cell lysis buffers that contain SDS such as RIPA buffer.**

Table 1. Materials Included with Kit. Store all materials at 4°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 -Detection 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°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 with a protein blocker such as .25% casein). 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. Avoid fingerprints, smudges, scratches, bubbles or liquid drops on the bottom and sides of the ELISA plate.
9. 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.
10. When calculating concentration of your sample from the assay, take into account the dilution factor.
11. 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. Do not prepare solutions in the ELISA plate.**

Assay procedure

1. Set aside and mark 12 wells for standard peptide dilutions. Configure six 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 a 225 pg/ml standard peptide.

Well	Standard Concentration	Standard Number	Dilution Instructions
1	150 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	150 pg/ml	2	Mix 100µl Standard Peptide with 50µl Standard Diluent. Remove 100µl to make standard 4.
3	100 pg/ml	3	Mix 100µl Standard Number 1 with 50µl Standard Diluent. Remove 100µl to make standard 5.
4	100 pg/ml	4	Mix 100µl Standard Number 2 with 50µl Standard Diluent. Remove 100µl to make standard 6.
5	50 pg/ml	5	Mix 100µl Standard Number 3 with100µl Standard Diluent. Remove 100µl to make standard 7. Remove 50µl, discard.
6	50 pg/ml	6	Mix 100µl Standard Number 4 with100µl Standard Diluent. Remove 100µl to make standard 8. Remove 50µl, discard.
7	25 pg/ml	7	Mix 100µl Standard Number 5 with 100µl Standard Diluent. Remove 100µl to make standard 9. Remove 50µl, discard.
8	25 pg/ml	8	Mix 100µl Standard Number 6 with 100µl Standard Diluent. Remove 100µl to make standard 10. Remove 50µl, discard.
9	12.5 pg/ml	9	Mix 100µl Standard Number 7 with 100µl Standard Diluent. Remove 50µl to make

			standard 11. Remove 100µl, discard.
10	12.5 pg/ml	10	Mix 100µl Standard Number 8 with 100µl Standard Diluent. Remove 50µl to make standard 12. Remove 100µl, discard.
11	6.2 pg/ml	11	Mix 50µl Standard Number 9 with 50µl Standard Diluent. Remove 50µl to make Standard .
12	6.2 pg/ml	12	Mix 50µl Standard Number 10 with 50µl Standard Diluent. Remove 50µl to make Standard

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: Set up two "blank wells:" Reproduce the ratio of sample vehicle (*without* sample) relative to sample diluent in the blank solution. The blank solution reproduces the content of the sample solution without the antigen. For each blank well, prepare 50 µl of mixed "blank solution."

   b) Standard solution wells: Prepare 50µl of standard at each dilution.

   c) Sample wells: For each well, prepare 50µl sample (that may have been previously diluted to meet the assay range).

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

   e) Immediately dispense 50 µl HRP-conjugate detection antibody solution (Component #4 Table 1) to each well. Then cover the plate with the closure membrane.

   f) Mix gently in a 37°C incubator for 30 min.

3. During the incubation period, prepare the wash solution: Dilute the 30X wash solution to 1X with $dH_2$0. Prepare 600ul 1X wash solution per well.

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. Repeat the wash steps 5 times with 30 second washes. Therefore 600ul wash solution is needed in total per well. 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 to each well. Gently mix solutions A and B. Incubate the plate 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 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

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Add samples to wells and HRP detection antibody conjugate, incubate for 30 min at RT at 37°C.

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Wash each well 6 times

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Add chromogen solutions A and B, 10 min at 37°C, dark

↓

Add stop solution

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Measure OD 450 nm within 10 min