BioVendor
Research
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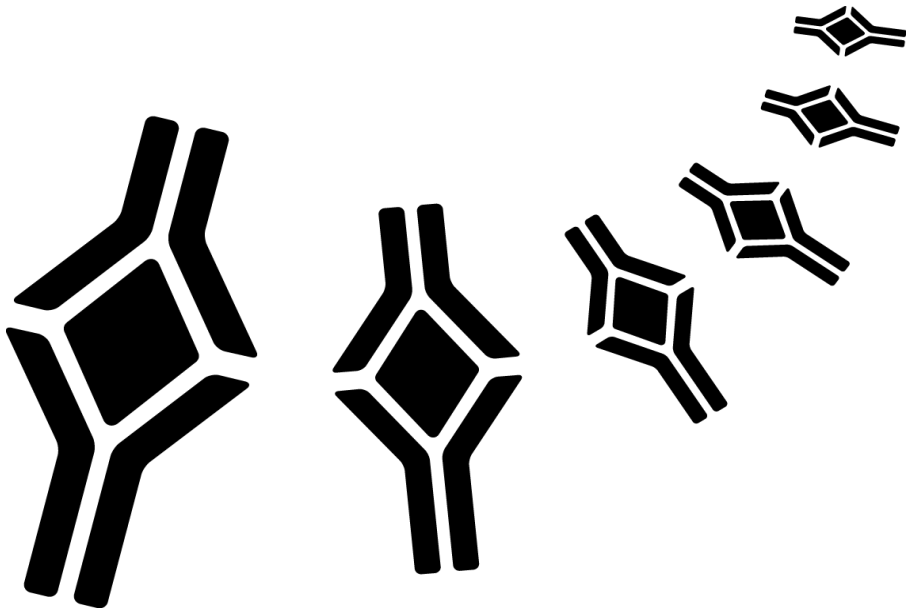


Human Amyloid Precursor Protein ELISA

Product Data Sheet

Cat. No.: RIG019R

For Research Use Only

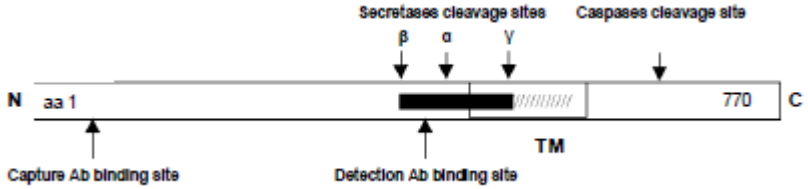
CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

Human APP ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of human amyloid precursor protein (APP) in human cerebral spinal fluid samples (CSF) and cell lysates. The assay recognizes both natural and recombinant human APP (including APP770, APP751, APP733, and APP695 isoforms), and may also detect mouse APP. APP is cleaved by b-secretase into a soluble sAPPb fragment and a C99 fragment. C99 is cleaved by γ-secretase to produce insoluble amyloid b (Ab) peptide. APP is also cleaved by a-secretase into a soluble sAPPa fragment and a C83 fragment. The capture antibody for the kit binds the N-terminus of human APP, while the detection antibody binds the N-terminus of the Ab peptide. The assays detects sAPPa, but not sAPPb.



CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.
Note: For safety and biohazard guidelines read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

2. REAGENTS PROVIDED

1 **Antibody Coated Wells,** 96-well plate
2 **Hu APP Standard**, lyophilized; contains 0.1% sodium azide
1 vial (25 ml) **Standard Diluent Buffer;** contains 0.1% sodium azide
1 vial (11 ml) Hu APP **Biotin Conjugate**; contains 0.1% sodium azide
1 vial (0,125 ml) **Streptavidin HRP (100X);** contains 3.3 mM thymol
1 vial (25 ml) **Streptavidin HRP Diluent;** contains 3.3 mM thymol
1 bottle (100 ml) **Wash Buffer Concentrate (25X)**
1 vial (25 ml) **Stabilized Chromogen, Tetramethylbenzidine (TMB)**
1 vial (25 ml) **Stop Solution**
3 **Adhesive Films**

3. STORAGE INSTUCTIONS – ELISA KIT

Upon receipt, store the kit at 2°C to 8°C.

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Cell Extraction Buffer

5. PRECAUCIONS FOR USE

- IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- Review the Sample Preparation and Handling in Documents available at Biovendor.com
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

6. PREPARATION OF REAGENTS

Preparation of 1x Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

7. PREPARATION OF SAMPLES

- **Prepare Cell Extraction Buffer**

Note: See the Sample Preparation and Handling in Documents available at Biovendor.com for detailed information on preparing Cell Extraction Buffer.

- Cell Extraction Buffer consists of 10 mM Tris (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM Na4P2O7, 2 mM Na3VO4, 1% Triton™ X-100, 10% glycerol, 0.1% SDS, and 0.5% deoxycholate.
- Immediately before use, add PMSF (0.3 M stock in DMSO) to 1 mM and 50 µL protease inhibitor cocktail for each 1 mL of Cell Extraction Buffer.

- **Prepare cell lysate**

- Collect cells by centrifugation (non-adherent cells) or scraping from culture flasks (adherent cells), then wash cells twice with cold PBS.
- Remove and discard the supernatant and collect the cell pellet. The pellet can be stored at −80°C and lysed at a later date if desired.
- Lyse the cell pellet in Cell Extraction Buffer for 30 minutes, on ice. Vortex at 10-minute intervals.

Note: The volume of Cell Extraction Buffer used depends on the number of cells in the cell pellet, and expression levels of human APP. FOR EXAMPLE, 108 HeLa cells can be extracted in 1 mL of Cell Extraction Buffer. Researchers must optimize the extraction procedures for their own applications.

- Transfer the lysate into microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.

- Transfer the supernatant into clean microcentrifuge tubes. Samples can be stored at −80°C (avoid multiple freeze-thaw cycles).

- **Pre-dilute samples**

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

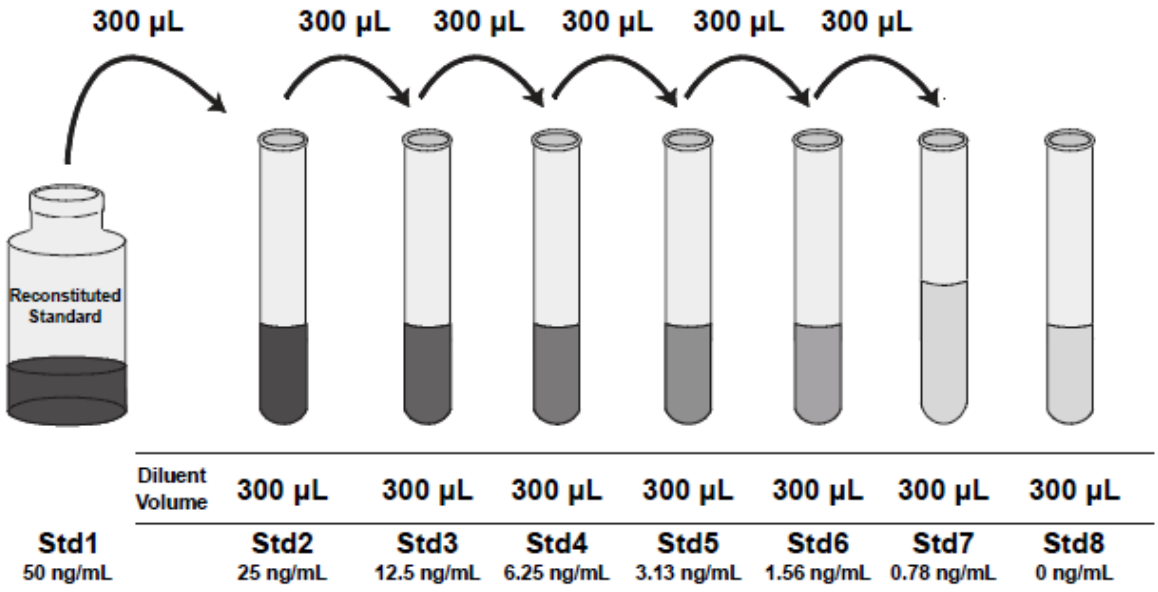
- Dilute samples prepared in Cell Extraction Buffer 1:10 or greater in Standard Diluent Buffer (e.g., 10 µL sample into 90 µL buffer). This dilution is necessary to reduce the matrix effect of the Cell Extraction Buffer. While a 1:10 sample dilution has been found to be satisfactory, higher dilutions such as 1:50 or 1:100 may be optimal. For 108 HeLa cells use 1–10 µL of the clarified lysate diluted to 100 µL in Standard Diluent Buffer for each well.

- Dilute samples >50 ng/mL with Standard Diluent Buffer.

- **Diluted Standards**

Note: Use glass or plastic tubes for diluting standards.
The Hu APP Standard was prepared using purified soluable APPa expressed in E. coli. It was calibrated against the mass of a highly purified recombinant human APP.

1. Reconstitute Hu APP Standard to 50 ng/mL Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 50 ng/mL human APP. Use the standard within 1 hour of reconstitution.
2. Add 600 µL Reconstituted Standard to a tube. Label as 50 ng/mL human APP.
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0 ng/mL human APP.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Remaining reconstituted standard should be discarded or frozen in aliquots at – 80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Prepare 1X Streptavidin-HRP Solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.
The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

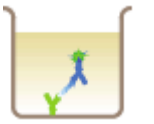
8. TEST PROTOCOL

• IMPORTANT! Perform a standard curve with each assay.
• Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
• Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

Bind antigen

a) Add 100 µL of standards, controls, or samples to the appropriate wells. Leave the wells for chromogen blanks empty.
b) Cover the plate with a plate cover and incubate for 2 hours at room temperature.
c) Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add Biotin-Conjugate



a) Add 100 µL Hu APP Biotin Conjugate solution into each well except the chromogen blanks.
b) Cover the plate with plate cover and incubate for 1 hour at room temperature.
c) Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add Streptavidin-HRP

a) Add 100 µL 1X Streptavidin-HRP solution into each well except the chromogen blanks.
b) Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c) Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

Add Stabilized Chromogen

a) Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b) Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

9. CALCULATION OF RESULTS

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

10. PERFORAMNCE CHARACTERISTICS

Standard curve (example)
The following data were obtained for the various standards over the range of 0 to 50 ng/mL human APP.

Standard Human APP (ng/mL)	Optical Density (450 nm)
50	3,34
25	2,10
12,5	1,29
6,25	0,71
3,13	0,43
1,56	0,31
0,78	0,23
0	0,12

Expected values
Twenty human CSF samples from normal individuals were evaluated using this assay. The APP values ranged from 245 to 3206 ng/mL (mean 1278 ng/mL).

Inter-assay precision
Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	22,12	13,38	3,12
Standard Deviation	1,16	1,18	0,25
% Coefficient of Variation	5,24	8,78	8,15

Intra-assay precision

Samples of known human APP concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	21,89	12,68	3,11
Standard Deviation	0,81	1,01	0,22
% Coefficient of Variation	3,72	7,99	7,05

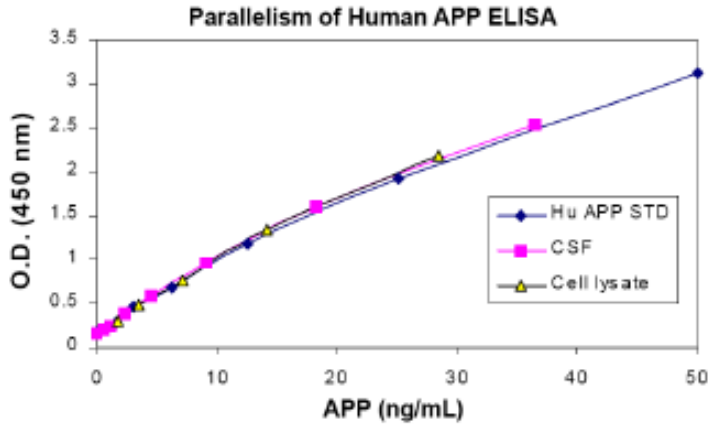
Linearity of dilution

Human CSF was serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Measured (ng/mL)	Expected (ng/mL)	Expected %
1/5	35,9	35,9	100
1/10	19,1	18,0	106,4
1/20	9,8	9,0	108,5
1/40	4,8	4,5	105,7
1/80	2,3	2,2	102,9

Parallelism

Natural human APP from APP-transfected CAD cells and human CSF was serially diluted in the Standard Diluent Buffer. The optical density of each dilution was plotted against the human APP standard curve. Parallelism demonstrates that the standard accurately reflects the human APP content in samples.



Recovery

To evaluate recovery, recombinant human APP was spiked into human CSF and then diluted 1:100 with Standard Diluent Buffer. The percent recovery over endogenous levels was calculated. On average, 112% recovery was observed.

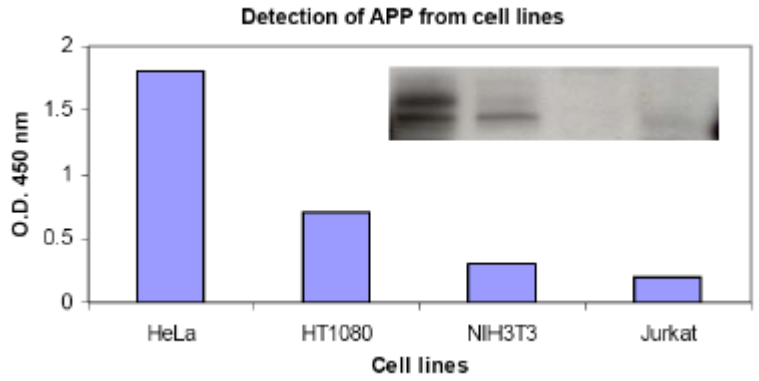
Sensitivity

The analytical sensitivity of this assay is <0.4 ng/mL human APP. This was determined by adding two standard deviations to the mean O.D. obtained from 30 assays of the zero standard. The sensitivity of the ELISA is ~8-fold greater than that of western blot when tested against known quantities of human APP.

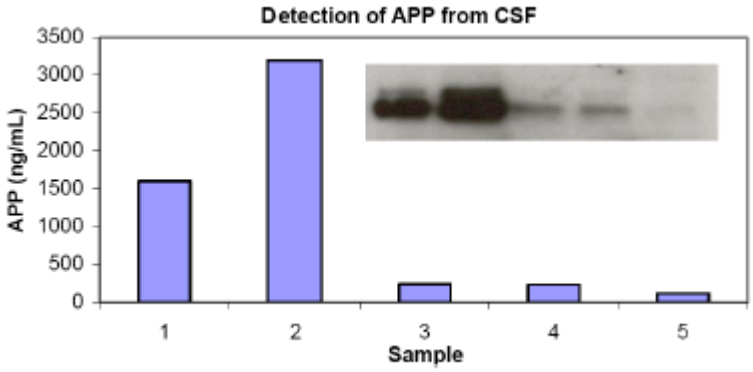
ELISA: OD 450 nm	0.134	0.211	0.311	0.423	0.694	1.339	2.140	3.430
APP (ng/test)	0	0.08	0.16	0.32	0.63	1.25	2.5	5

Specificity

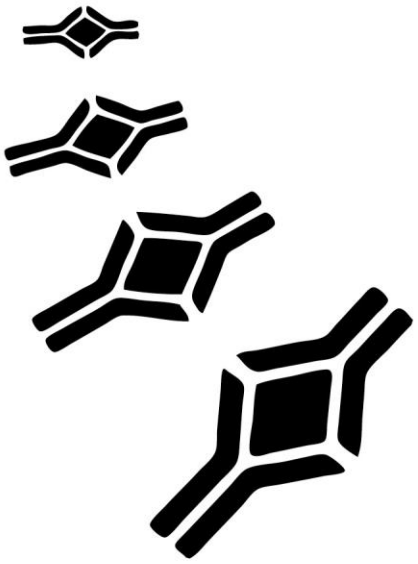
The Human APP ELISA Kit is specific for measurement of total APP protein. The following proteins were found to have no cross-reactivity at 100 ng/mL: tau, a-synuclein, b-synuclein, Ab1-40, and Ab1-42. Cell extracts from several cell lines (200 µg/mL protein) was analyzed by ELISA and western blot to confirm kit specificity. The data show that levels of human APP protein are consistent when detected with either the ELISA kit or by western blot (inset in graph).



Cell extracts from human cerebral spinal fluid (1:16 dilution) was analyzed by ELISA and western blot to confirm kit specificity. The data show that levels of human APP protein are consistent when detected with either the ELISA kit or by western blot (inset in graph).



Notes



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