Human APP ELISA Kit

Catalog Number KHB0051 (96 tests)

Pub. No. MAN0014876 **Rev.** 4.0 (32)

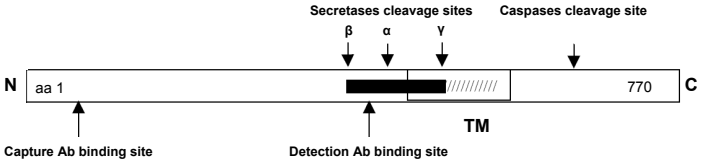
CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous 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, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ 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 APP_{770}, APP_{751}, APP_{733}, and APP_{695} isoforms), and may also detect mouse APP.

APP is cleaved by β-secretase into a soluble sAPPβ fragment and a C99 fragment. C99 is cleaved by γ-secretase to produce insoluble amyloid β (Aβ) peptide. APP is also cleaved by α-secretase into a soluble sAPPα 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 Aβ peptide. The assays detects sAPPα, but not sAPPβ.



Contents and storage

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

Contents	Cat. No. KHB0051 (96 tests)
Hu APP Standard, lyophilized; contains 0.1% sodium azide	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
Antibody Coated Wells, 96-well plate	1 plate
Hu APP Biotin Conjugate; contains 0.1% sodium azide	11 mL
Streptavidin HRP (100X)	0.15 mL
Streptavidin HRP Diluent; contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

Required materials not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Cell Extraction Buffer (Cat. No. FNN0011, or see "Prepare Cell Extraction Buffer")

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 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.

Prepare Cell Extraction Buffer

Note: See the *ELISA Technical Guide* for detailed information on preparing Cell Extraction Buffer.

1. Prepare 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 $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton™ X-100, 10% glycerol, 0.1% SDS, and 0.5% deoxycholate.
2. Immediately before use, add PMSF (0.3 M stock in DMSO) to 1 mM and 50 µL protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714) for each 1 mL of Cell Extraction Buffer.

Prepare cell lysate

1. Collect cells by centrifugation (non-adherent cells) or scraping from culture flasks (adherent cells), then wash cells twice with cold PBS.
2. 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.
3. 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, 10^8 HeLa cells can be extracted in 1 mL of Cell Extraction Buffer. Researchers must optimize the extraction procedures for their own applications.
4. Transfer the lysate into microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
5. 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.

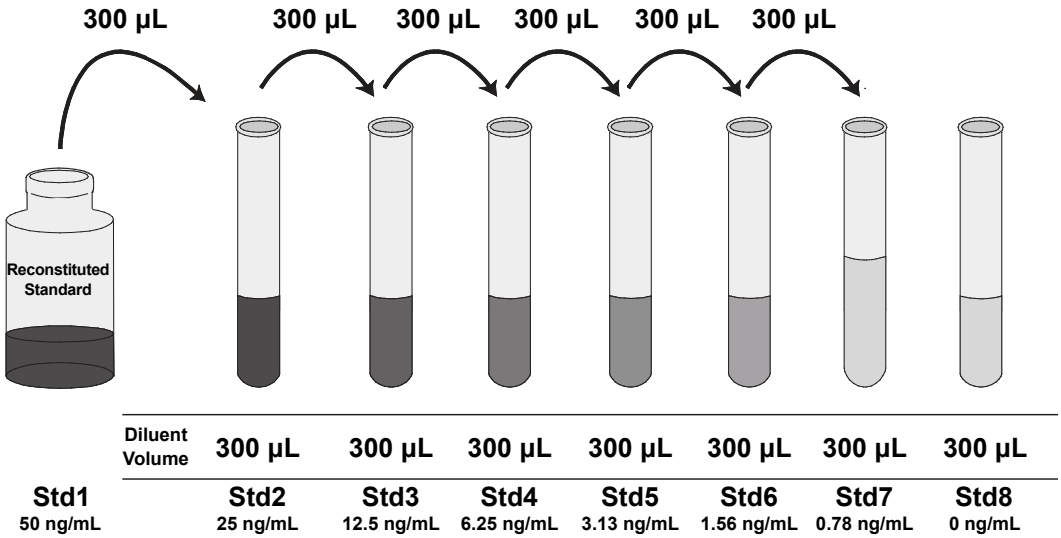
  For 10^8 HeLa cells use 1–10 µL of the clarified lysate diluted to 100 µL in Standard Diluent Buffer for each well.
- Dilute samples >50 ng/mL with Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

The Hu APP Standard was prepared using purified soluable APPα 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.

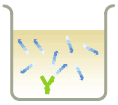
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

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.

1 Bind antigen



a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) 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.

2 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.

3 Add Streptavidin-HRP

a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) 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.

4 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.

5 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.

Read the plate and generate the standard curve

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.

Performance characteristics

Standard curve example

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

Std. 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

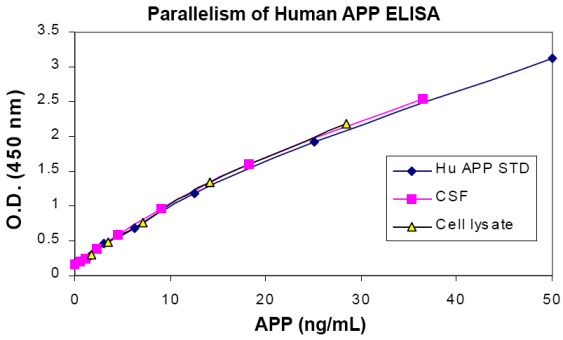
Linearity of dilution

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

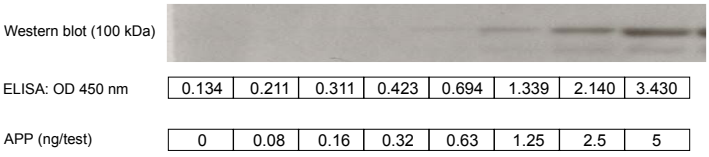
Natural human APP from APP-transfected CAD cells and human CSF was serially diluted in the Standard Diluent Buffer. The optical density of each dilution was plotted against the human APP standard curve. Parallelism demonstrates that the standard accurately reflects the human APP content in samples.



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

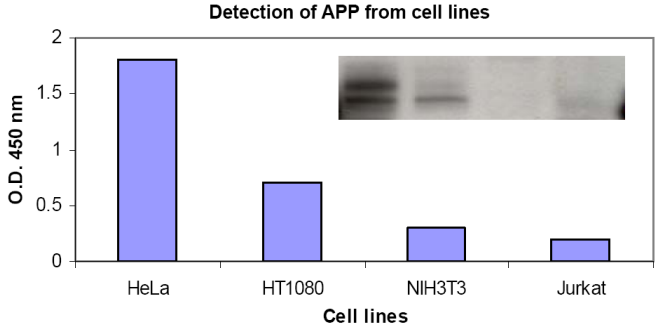
Recovery

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.

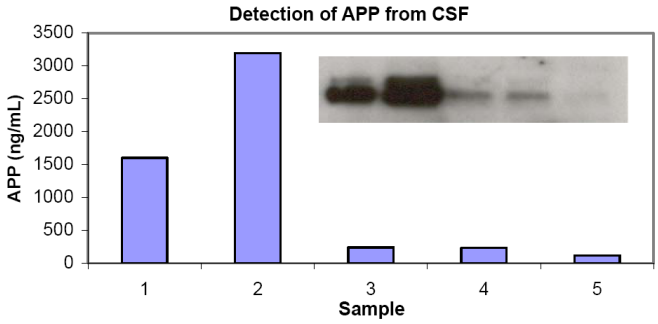
Specificity

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, α-synuclein, β-synuclein, Aβ1-40, and Aβ1-42.

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).



Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.