ab326890 – Human Amyloid Precursor Protein SimpleStep ELISA® Kit - Chemiluminescent

For the quantitative measurement of Amyloid Precursor Protein in human serum, plasma, urine, cerebrospinal fluid, cell culture supernatants, cell and tissue extract samples.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab326890

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except typical standard curve and sensitivity, including native linearity, were collected using the colorimetric version of this kit ab216944.

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human Amyloid Precursor Protein Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human Amyloid Precursor Protein Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human Amyloid Precursor Protein Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 4BI	6 mL	10 x 6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	2 x 50 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	10 x 1 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
ChemiHRP Reagent A	3 mL	50 mL	+4°C
ChemiHRP Reagent B	3 mL	50 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	10 x 96 Wells	+4°C
Plate Seal	1	10	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the **Amyloid Precursor Protein** protein standard by adding the volume indicated on the protein vial label. For **serum, plasma, cell culture supernatant, cerebrospinal fluid, and urine samples measurements**, use Sample Diluent NS. For **cell and tissue extract samples measurements**, use 1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 13,330 pg/mL **Stock Standard #1** Solution.
2. Label eight tubes, Standards 1– 8.
3. Use the same Sample Diluent as used to resuspend the **Stock Standard #1** to prepare the standard curve. Add 150 µL of Sample Diluent into numbers 2-8.
4. Use the **Stock Standard #1** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard #1**	150	0	13,330	13,330
2	**Stock Standard #1**	75	150	13,330	4,443.3
3	Standard#2	75	150	4,443.3	1,481.1
4	Standard#3	75	150	1,481.1	493.7
5	Standard#4	75	150	493.7	164.57
6	Standard#5	75	150	164.57	54.86
7	Standard#6	75	150	54.86	18.29
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:160 – 1:10
Plasma – Citrate	1:160 – 1:10
Plasma – Heparin	1:160 – 1:5
Plasma – EDTA	1:160 – 1:10
Cerebrospinal Fluid	1:6,400 – 1:200
Urine	1:128 – 1:4
A-549 Cell Culture Supernatant	1:64 – 1:4
HeLa Cell Culture Supernatant (3 days)	1:128 – 1:4
Brain Tissue Extract	0.16 – 20 µg/mL
Liver Tissue Extract	7.81 – 500 µg/mL
SH-SY5Y Cell Extract	6.25 – 100 µg/mL
U-87 MG Cell Extract	3.13 – 100 µg/mL
HeLa Cell Extract	0.78 – 25 µg/mL

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:10 into Sample Diluent NS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:10 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Δ Note: This assay cross-reacts with Amyloid Precursor Protein present in bovine serum, a common additive of cell culture media. To assay supernatants, it is recommended to use serum free media or assay the cell culture media containing bovine serum in parallel with the supernatants and then subtract the media signal.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:4 into Sample Diluent NS and assay. Store un-diluted urine samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Cerebrospinal Fluid (CSF) Dilute samples at least 1:200 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Preparation of extracts from cell pellets Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4ºC. Rinse cells twice with PBS. Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from adherent cells by direct lysis (alternative protocol) Remove growth media and rinse adherent cells 2 times in PBS. Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate). Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from tissue homogenates Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 750 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support
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Version 2a | 2026-04-13

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human Amyloid Precursor Protein.

This kit recognizes a N-APP (amino acid 18-286 of human Amyloid Precursor Protein) and Soluble APP-alpha (amino acid 18-687 of human Amyloid Precursor Protein).

The standard protein in this kit is an N-terminal fragment human Amyloid Precursor Protein.

Native signal was detected in serum, plasma (citrate, EDTA, heparin), cell culture supernatant, urine, CSF, cell extract, and tissue extract sample types.

Saliva and milk samples have not been tested with this kit.

SPECIES REACTIVITY

Other species reactivity was determined by measuring neat serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity was determined for the following species: Cow (4%), Mouse (<3%), Rat (<3%).

Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most chemiluminescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted chemiluminescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed

Standard Curve Measurements			
Concentration (pg/mL)	**RLU**		**Mean RLU**
	1	**2**	
0	2,737	2,737	2,737
18.29	4,319	4,328	4,324
54.86	7,288	7,033	7,161
164.57	13,938	14,194	14,066
493.7	39,599	36,060	37,830
1,481.1	124,157	123,274	123,716
4,443.3	327,424	339,210	333,317
13,330	1,028,324	1,066,292	1,047,308

Table 1. Example of human Amyloid Precursor Protein standard curve in Sample Diluent NS. The Amyloid Precursor Protein standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	**RLU**		**Mean RLU**
	1	**2**	
0	2,795	2,795	2,795
18.29	4,150	3,739	3,945
54.86	6,109	5,904	6,007
164.57	12,131	12,009	12,070
493.7	31,268	27,796	29,532
1,481.1	95,325	86,947	91,136
4,443.3	244,162	253,024	248,593
13,330	769,817	783,232	776,525

Table 2. Example of human Amyloid Precursor Protein standard curve in 1X Cell Extraction Buffer PTR. The Amyloid Precursor Protein standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent NS	12	3.13 pg/mL
1X Cell Extraction Buffer PTR	12	5.45 pg/mL

Recovery

Three concentrations of Amyloid Precursor Protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
2% Serum	112	111 – 114
2% Plasma Citrate	91	85 – 105
2% Plasma Heparin	106	104 – 109
2% Plasma EDTA	109	93 – 123
0.25% Cerebrospinal Fluid	101	92 – 107
12.5% Urine	93	86 – 100
5% A-549 Cell Culture Supernatant	112	107 – 117
5% HeLa Cell Culture Supernatant (3 days)	109	100 – 119
5 µg/mL Brain Tissue Extract	112	107 – 118
125 µg/mL Liver Tissue Extract	87	81 – 93
25 µg/mL SH-SY5Y Cell Extract	93	90 – 97
25 µg/mL U-87 MG Cell Extract	102	94 – 117
5 µg/mL HeLa Cell Extract	100	97 – 103

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Amyloid Precursor Protein was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	10% Human Serum	10% Human Plasma (Citrate)	10% Human Plasma (EDTA)	20% Human Plasma (Heparin)
Undiluted	pg/mL	5,610	6,226	3,838	6,053
	% Expected value	100	100	100	100
2	pg/mL	2,537	3,139	1,802	3,246
	% Expected value	90	101	94	107
4	pg/mL	1,299	1,596	859.5	1,501
	% Expected value	93	103	90	99
8	pg/mL	656.1	814.7	451.4	788.8
	% Expected value	94	105	94	104
16	pg/mL	333.9	408.3	238.2	405.1
	% Expected value	95	105	99	107

Native Amyloid Precursor Protein was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS. Sample abbreviations: cell culture supernatant (SN), cerebrospinal fluid (CSF).

Dilution Factor	Interpolated value	0.5% Human CSF	25% Human Urine	25% A-549 SN	25% HeLa SN (3 days)
Undiluted	pg/mL	3,222	2,253	6,284	1,931
	% Expected value	100	100	100	100
2	pg/mL	1,493	1,142	2,963	884.3
	% Expected value	93	101	94	92
4	pg/mL	708.8	580.7	1,386	433.8
	% Expected value	88	103	88	90
8	pg/mL	341	296	721.8	217.2
	% Expected value	85	105	92	90
16	pg/mL	183	141.1	352.8	108.9
	% Expected value	91	100	90	90

Native Amyloid Precursor Protein was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	100 µg/mL SH-SY5Y Cell Extract	100 µg/mL U-87 MG Cell Extract	25 µg/mL HeLa Cell Extract	20 µg/mL Brain Tissue Extract	500 µg/mL Liver Tissue Extract
Undiluted	pg/mL	5,858	5,611	1,290	4,574	4,446
	% Expected value	100	100	100	100	100
2	pg/mL	3,013	2,850	648.6	2,069	1,938
	% Expected value	103	102	101	90	87
4	pg/mL	1,485	1,402	303.4	1,019	1,011
	% Expected value	101	100	94	89	91
8	pg/mL	760.8	697.9	146.6	504.5	534.7
	% Expected value	104	100	91	88	96
16	pg/mL	393.4	367.3	66.41	274.1	279.6
	% Expected value	107	105	82	96	101

Precision

Mean coefficient of variations of interpolated values of Amyloid Precursor Protein from three concentrations of human serum within the working range of the assay.

	Intra-assay	Inter-assay
n=	3	5
CV (%)	6.2	5.1

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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Version 2a | 2026-04-13

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