abcam

Product datasheet

Human Amyloid Precursor Protein ELISA Kit ab216944

יילשעבע SimpleStep ELISA

画像数 10

製品の概要

製品名

Human Amyloid Precursor Protein ELISA Kit

検出方法

Colorimetric

再現性

Intra-Assay(同時再現性)

サンプル	N	平均値	SD	CV%	
Serum	3			6.2%	

Inter-Assay(日差再現性)

サンプル	N	平均值	SD	CV%
Serum	5			5.1%

サンプルの種類

Cell culture supernatant, Urine, Serum, Cell culture extracts, Tissue Extracts, Hep Plasma, EDTA Plasma, Cit plasma, Cerebral Spinal Fluid

アッセイタイプ

Sandwich (quantitative)

検出感度

14.9 pg/ml

検出範囲

93.75 pg/ml - 6000 pg/ml

添加回収試験

特定サンプルでの回収試験

サンプルの種類	平均 %	測定範囲
Cell culture supernatant	112	107% - 117%
Urine	93	86% - 100%
Serum	112	111% - 114%
Cell culture extracts	93	90% - 97%
Tissue Extracts	112	107% - 118%
Hep Plasma	106	104% - 109%

サンプルの種類	平均 %	測定範囲
EDTA Plasma	109	93% - 123%
Cit plasma	91	85% - 105%
Cerebral Spinal Fluid	101	92% - 107%

全工程の試験時間

1h 30m

ステップ

One step assay

種交差性

交差種: Human

製品の概要

Human Amyloid Precursor Protein ELISA Kit (ab216944) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of Amyloid Precursor Protein protein in cell culture extracts, cell culture supernatant, cerebral spinal fluid, cit plasma, edta plasma, hep plasma, serum, tissue extracts, and urine. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human Amyloid Precursor Protein with 14.9 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (<u>ab203359</u>) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

Amyloid Precursor Protein (GeneCards: APP) is a multifunctional transmembrane protein that consists of a 682 amino acid (aa) long extracellular domain, a 24 aa long transmembrane segment, and a 47 aa long cytoplasmic domain. Alternative splicing generates multiple isoforms including the most prevalent APP695, APP751, and APP770. Isoform APP695 is the predominant form in neuronal tissue, isoform APP751 and isoform APP770 are widely expressed in non-neuronal cells. Isoform APP751 is the most abundant form in T-lymphocytes. Amyloid Precursor Protein functions as a cell surface receptor and performs physiological functions on the surface of neurons relevant to neurite growth, neuronal adhesion and axonogenesis. Amyloid Precursor Protein is involved in cell mobility and transcription regulation through protein-protein interactions.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

試験プラットフォーム

Pre-coated microplate (12 x 8 well strips)

特記事項

保存方法

Store at +4°C. Please refer to protocols.

内容	1 x 96 tests
10X Human Amyloid Precursor Protein Capture Antibody	1 x 600µl
10X Human Amyloid Precursor Protein Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent 4BI	1 x 6ml
Human Amyloid Precursor Protein Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 50ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

機能

Functions as a cell surface receptor and performs physiological functions on the surface of neurons relevant to neurite growth, neuronal adhesion and axonogenesis. Involved in cell mobility and transcription regulation through protein-protein interactions. Can promote transcription activation through binding to APBB1-KAT5 and inhibits Notch signaling through interaction with Numb. Couples to apoptosis-inducing pathways such as those mediated by G(O) and JIP. Inhibits G(o) alpha ATPase activity (By similarity). Acts as a kinesin I membrane receptor, mediating the axonal transport of beta-secretase and presenilin 1. Involved in copper homeostasis/oxidative stress through copper ion reduction. In vitro, copper-metallated APP induces neuronal death directly or is potentiated through Cu(2+)-mediated low-density lipoprotein oxidation. Can regulate neurite outgrowth through binding to components of the extracellular matrix such as heparin and collagen I and IV. The splice isoforms that contain the BPTI domain possess protease inhibitor activity. Induces a AGER-dependent pathway that involves activation of p38 MAPK, resulting in internalization of amyloid-beta peptide and leading to mitochondrial dysfunction in cultured cortical neurons. Provides Cu(2+) ions for GPC1 which are required for release of nitric oxide (NO) and subsequent degradation of the heparan sulfate chains on GPC1.

Beta-amyloid peptides are lipophilic metal chelators with metal-reducing activity. Bind transient metals such as copper, zinc and iron. In vitro, can reduce Cu(2+) and Fe(3+) to Cu(+) and Fe(2+), respectively. Beta-amyloid 42 is a more effective reductant than beta-amyloid 40. Beta-amyloid peptides bind to lipoproteins and apolipoproteins E and J in the CSF and to HDL particles in plasma, inhibiting metal-catalyzed oxidation of lipoproteins. Beta-APP42 may activate mononuclear phagocytes in the brain and elicit inflammatory responses. Promotes both tau aggregation and TPK II-mediated phosphorylation. Interaction with overexpressed HADH2 leads to oxidative stress and neurotoxicity. Also binds GPC1 in lipid rafts.

Appicans elicit adhesion of neural cells to the extracellular matrix and may regulate neurite outgrowth in the brain.

The gamma-CTF peptides as well as the caspase-cleaved peptides, including C31, are potent enhancers of neuronal apoptosis.

N-APP binds TNFRSF21 triggering caspase activation and degeneration of both neuronal cell bodies (via caspase-3) and axons (via caspase-6).

Expressed in all fetal tissues examined with highest levels in brain, kidney, heart and spleen. Weak expression in liver. In adult brain, highest expression found in the frontal lobe of the cortex and in the anterior perisylvian cortex-opercular gyri. Moderate expression in the cerebellar cortex, the posterior perisylvian cortex-opercular gyri and the temporal associated cortex. Weak expression found in the striate, extra-striate and motor cortices. Expressed in cerebrospinal fluid, and plasma. Isoform APP695 is the predominant form in neuronal tissue, isoform APP751 and isoform APP770 are widely expressed in non-neuronal cells. Isoform APP751 is the most abundant form in T-lymphocytes. Appican is expressed in astrocytes.

Alzheimer disease 1

Cerebral amyloid angiopathy, APP-related

Belongs to the APP family.

Contains 1 BPTI/Kunitz inhibitor domain.

The basolateral sorting signal (BaSS) is required for sorting of membrane proteins to the basolateral surface of epithelial cells.

The NPXY sequence motif found in many tyrosine-phosphorylated proteins is required for the specific binding of the PID domain. However, additional amino acids either N- or C-terminal to the NPXY motif are often required for complete interaction. The PID domain-containing proteins which bind APP require the YENPTY motif for full interaction. These interactions are independent of phosphorylation on the terminal tyrosine residue. The NPXY site is also involved in clathrin-mediated endocytosis.

Proteolytically processed under normal cellular conditions. Cleavage either by alpha-secretase, beta-secretase or theta-secretase leads to generation and extracellular release of soluble APP peptides, S-APP-alpha and S-APP-beta, and the retention of corresponding membrane-anchored C-terminal fragments, C80, C83 and C99. Subsequent processing of C80 and C83 by gamma-secretase yields P3 peptides. This is the major secretory pathway and is non-amyloidogenic. Alternatively, presenilin/nicastrin-mediated gamma-secretase processing of C99 releases the amyloid beta proteins, amyloid-beta 40 (Abeta40) and amyloid-beta 42 (Abeta42), major components of amyloid plaques, and the cytotoxic C-terminal fragments, gamma-CTF(50), gamma-CTF(57) and gamma-CTF(59). Many other minor beta-amyloid peptides, beta-amyloid 1-X peptides, are found in cerebral spinal fluid (CSF) including the beta-amyloid X-15 peptides, produced from the cleavage by alpha-secretase and all terminating at Gln-686.

Proteolytically cleaved by caspases during neuronal apoptosis. Cleavage at Asp-739 by either caspase-6, -8 or -9 results in the production of the neurotoxic C31 peptide and the increased production of beta-amyloid peptides.

N- and O-glycosylated. O-glycosylation on Ser and Thr residues with core 1 or possibly core 8 glycans. Partial tyrosine glycosylation (Tyr-681) is found on some minor, short beta-amyloid peptides (beta-amyloid 1-15, 1-16, 1-17, 1-18, 1-19 and 1-20) but not found on beta-amyloid 38, beta-amyloid 40 nor on beta-amyloid 42. Modification on a tyrosine is unusual and is more prevelant in AD patients. Glycans had Neu5AcHex(Neu5Ac)HexNAc-O-Tyr, Neu5AcNeu5AcHex(Neu5Ac)HexNAc-O-Tyr and O-AcNeu5AcNeu5AcHex(Neu5Ac)HexNAc-O-Tyr structures, where O-Ac is O-acetylation of Neu5Ac. Neu5AcNeu5Ac is most likely Neu5Ac 2,8Neu5Ac linked. O-glycosylations in the vicinity of the cleavage sites may influence the

proteolytic processing. Appicans are L-APP isoforms with O-linked chondroitin sulfate.

Phosphorylation in the C-terminal on tyrosine, threonine and serine residues is neuron-specific.

組織特異性

関連疾患

配列類似性

ドメイン

翻訳後修飾

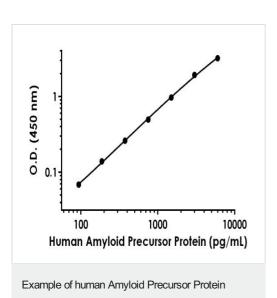
Phosphorylation can affect APP processing, neuronal differentiation and interaction with other proteins. Phosphorylated on Thr-743 in neuronal cells by Cdc5 kinase and Mapk10, in dividing cells by Cdc2 kinase in a cell-cycle dependent manner with maximal levels at the G2/M phase and, in vitro, by GSK-3-beta. The Thr-743 phosphorylated form causes a conformational change which reduces binding of Fe65 family members. Phosphorylation on Tyr-757 is required for SHC binding. Phosphorylated in the extracellular domain by casein kinases on both soluble and membrane-bound APP. This phosphorylation is inhibited by heparin.

Extracellular binding and reduction of copper, results in a corresponding oxidation of Cys-144 and Cys-158, and the formation of a disulfide bond. In vitro, the APP-Cu(+) complex in the presence of hydrogen peroxide results in an increased production of beta-amyloid-containing peptides. Trophic-factor deprivation triggers the cleavage of surface APP by beta-secretase to release sAPP-beta which is further cleaved to release an N-terminal fragment of APP (N-APP). Beta-amyloid peptides are degraded by IDE.

Membrane. Membrane, clathrin-coated pit. Cell surface protein that rapidly becomes internalized via clathrin-coated pits. During maturation, the immature APP (N-glycosylated in the endoplasmic reticulum) moves to the Golgi complex where complete maturation occurs (O-glycosylated and sulfated). After alpha-secretase cleavage, soluble APP is released into the extracellular space and the C-terminal is internalized to endosomes and lysosomes. Some APP accumulates in secretory transport vesicles leaving the late Golgi compartment and returns to the cell surface. Gamma-CTF(59) peptide is located to both the cytoplasm and nuclei of neurons. It can be translocated to the nucleus through association with APBB1 (Fe65). Beta-APP42 associates with FRPL1 at the cell surface and the complex is then rapidly internalized. APP sorts to the basolateral surface in epithelial cells. During neuronal differentiation, the Thr-743 phosphorylated form is located mainly in growth cones, moderately in neurites and sparingly in the cell body. Casein kinase phosphorylation can occur either at the cell surface or within a post-Golgi compartment. Associates with GPC1 in perinuclear compartments. Colocalizes with SORL1 in a

細胞内局在

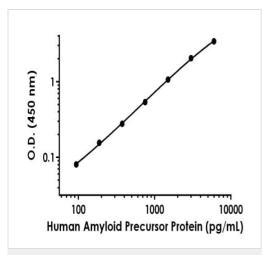
画像



standard curve in Sample Diluent NS.

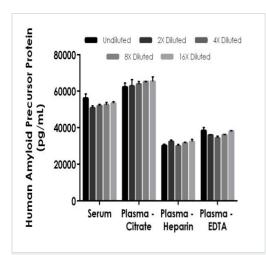
Background-subtracted data values (mean +/- SD) are graphed.

vesicular pattern in cytoplasm and perinuclear regions.



Example of human Amyloid Precursor Protein standard curve in 1X Cell Extraction Buffer PTR.

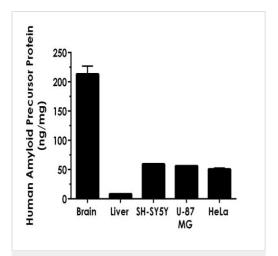




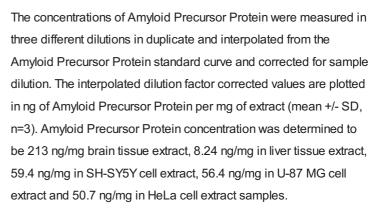
Interpolated concentrations of native Amyloid

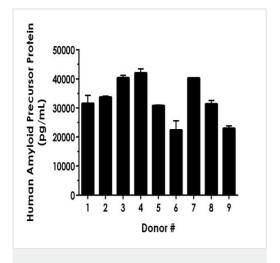
Precursor Protein in human serum and plasma
samples.

The concentrations of Amyloid Precursor Protein were measured in duplicates, interpolated from the Amyloid Precursor Protein standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 10%, plasma (citrate) 10%, plasma (heparin) 20% and plasma (EDTA) 10%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Amyloid Precursor Protein concentration was determined to be 52,942 pg/mL in neat serum, 63,882 pg/mL in neat plasma (citrate), 31,342 pg/mL in neat plasma (heparin), and 36,605 pg/mL in neat plasma (EDTA).



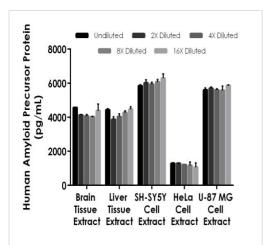
Interpolated concentrations of native Amyloid Precursor Protein in human extract samples.





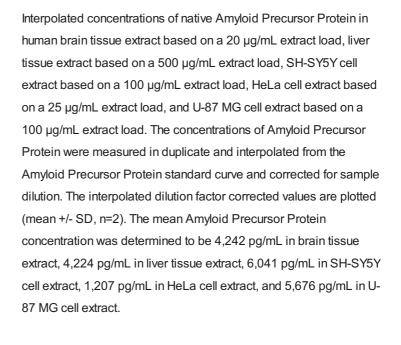
Serum from nine individual healthy human male donors was measured in duplicate.

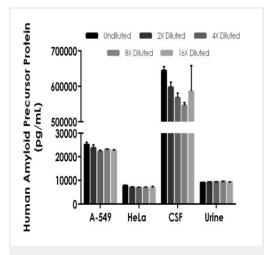
Interpolated dilution factor corrected values are plotted (mean +/-SD, n=2). The mean Amyloid Precursor Protein concentration was determined to be 32,874 pg/mL with a range of 22,456 – 42,097 pg/mL.



Interpolated concentrations of native Amyloid

Precursor Protein in human brain tissue extract, liver
tissue extract, SH-SY5Y cell extract, HeLa cell
extract and U-87 MG cell extract.





Interpolated concentrations of native Amyloid
Precursor Protein in human cerebrospinal fluid
(CSF), urine, A-549 cell culture supernatant, and
HeLa cell culture supernatant (3 days).

The concentrations of Amyloid Precursor Protein were measured in duplicates, interpolated from the Amyloid Precursor Protein standard curves and corrected for sample dilution. Undiluted samples are as follows: A-549 cell culture supernatant 25%, HeLa cell culture supernatant 25%, cerebrospinal fluid 0.5%, and urine 25%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Amyloid Precursor Protein concentration was determined to be 23,336 pg/mL in neat A-549 cell culture supernatant, 7,133 pg/mL in neat HeLa cell culture supernatant, 58,7958 pg/mL in neat cerebrospinal fluid, and 9,188 pg/mL in neat urine.



SimpleStep ELISA technology allows the formation of the antibodyantigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.

Sandwich ELISA - Human Amyloid Precursor Protein ELISA Kit (ab216944)

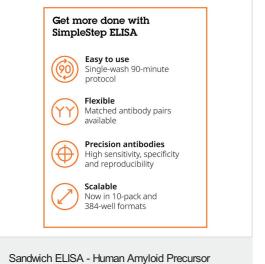


Sandwich ELISA - Human Amyloid Precursor

Protein ELISA Kit (ab216944)

Protein ELISA Kit (ab216944)

To learn more about the advantages of recombinant antibodies see **here**.



To learn more about the advantages of SimpleStep ELISA[®] kits see <u>here</u>.

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