Datasheet for ABIN579070

APP ELISA Kit

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Overview

Quantity:	96 tests
Target:	APP
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	0.312-20 ng/mL
Minimum Detection Limit:	0.312 ng/mL
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the specific measurement of rat amyloid beta peptide 1-42, Abeta1-42 concentrations in cell culture supernates, serum and plasma.
Sample Type:	Cell Culture Supernatant, Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural rat Abeta1-42.
Cross-Reactivity (Details):	No significant cross-reactivity or interference was observed.
Sensitivity:	0.11ng/ml
Characteristics:	Rattus norvegicus,Rat,Amyloid beta A4 protein,ABPP,APP,Alzheimer disease amyloid A4 protein homolog,Amyloidogenic glycoprotein,AG,App
Components:	Reagent (Quantity):

Product Details

	• Assay plate (1), • Standard (2), • Sample Diluent (1×20 mL), • Assay Diluent A (1×10 mL), • Assay Diluent B (1×10 mL), • Detection Reagent A (1×120 µL), • Detection Reagent B (1×120 µL), • Wash Buffer(25 x concentrate) (1×30 mL), • Substrate (1×10 mL), • 2 Stop Solution (1×10 mL), • Plate sealer for 96 wells (5), • Instruction (1)
Material not included:	Microplate reader. Pipettes and pipette tips. EP tube Deionized or distilled water.

Target Details

Target:	APP
Alternative Name:	App (APP Products)
Background:	One of the hallmarks of Alzheimer's disease is the self-aggregation of the amyloid beta peptide (Abeta) in extracellular amyloid fibrils. Abeta is a peptide composed of 40 to 42 (43) amino acids, and is said to be cleaved out of the precursor protein APP (a protein composed of 695, 751, or 770 amino acids) by the action of beta- or gamma-secretase.In addition, the presence of numerous variant A beta molecules has been demonstrated in the culture fluid of mouse neuroblastoma cells transfected with cDNA coding rat amyloid precursor protein (APP). Among the different forms of Abeta, the 42-residue fragment (Abeta1-42) readily self-associates and forms nucleation centers from where fibrils can quickly grow. The strong tendency of Abeta1-42 to aggregate is one of the reasons for the scarcity of data on its fibril formation process. Abeta1-40 is the most common form secreted from cultured cells and found in cerebro-spinal fluid (CSF). Abeta1-42 is the major component of senile plaques and considered as the most crucial factor in AD pathogenesis
Gene ID:	3039
Pathways:	Caspase Cascade in Apoptosis, EGFR Signaling Pathway, Transition Metal Ion Homeostasis, Skeletal Muscle Fiber Development, Toll-Like Receptors Cascades, Feeding Behaviour

Application Details

Sample Volume:	100 µL

Application Details

Plate:	Pre-coated
Protocol:	This assay employs the quantitative sandwich enzyme immunoassay technique. A antibody specific for Abeta1-42 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Abeta1-42 present is bound by the immobilized antibody. An enzyme-linked antibody specific for Abeta1-42 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Abeta1-42 bound in the initial step. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	Bring all reagents to room temperature before use. Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). The undiluted standard serves as the high standard. The Sample Diluent serves as the zero standard (0 ng/ml).
Sample Collection:	Cell culture supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20 °C. Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2 - 8 °C within 30 minutes of collection. Store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Note: Citrate plasma has not been validated for use in this assay.
Assay Procedure:	Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37 °C directly.). All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4 °C until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. 1. Add 100 µL of Standard, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37 °C .

2. Remove the liquid of each well, don ' t wash.

3. Add 100 µL of Detection Reagent A working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37 °C . Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100 µL of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for 1 hours at 37 °C .

6. Repeat the aspiration/wash as in step 4.

7. Add 90 µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate within 30 minutes at 37 °C . Protect from light.

8. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

Important Note:

1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption.

2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10 µ l for once pipetting.

3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the 5 strips DRY at any time during the assay.

4. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.

5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

Application Details

	6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. 7. Duplication of all standards and specimens, although not required, is recommended. 8. Substrate Solution is easily contaminated. Please protect it from light.
Calculation of Results:	Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the SAA concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 13.0. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Restrictions:	For Research Use only

Handling

Handling Advice:	1. The kit should not be used beyond the expiration date on the kit label. 2. Do not mix or substitute reagents with those from other lots or sources. 3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique,incubation time or temperature, and kit age can cause variation in binding. 4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	The Assay Plate, Standard, Detection Reagent A and Detection Reagent B should be stored at -20°C upon being received. After receiving the kit , Substrate should be always stored at 4°C.

Publications

Product cited in:	Grolla, Fakhfouri, Balzaretti, Marcello, Gardoni, Canonico, DiLuca, Genazzani, Lim: "Aβ leads to Ca^{2+} signaling alterations and transcriptional changes in glial cells." in: **Neurobiology of aging**,

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