Affinity-purified Rabbit Anti-human APP^{+1} Antibody

ORDERING INFORMATION

Catalog Number: AF850

Lot Number: CTM02

Size: 50 µg

Formulation: 0.2 µm filtered solution in PBS

Storage: -20° C

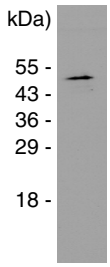
Reconstitution: sterile PBS

Specificity: human APP^{+1}

Immunogen: human APP^{+1}

Ig Type: rabbit IgG

Application: Western blot



Immunoblot of SDS-solubilized partially purified recombinant human APP^{+1}. Samples were electrophoresed on 15% gels and immunoblotting was with 0.5 µg/mL anti-APP^{+1}. A one minute exposure is shown.

Preparation

Rabbits were immunized with the KLH coupled synthetic peptide CMRMGRGRTSSKELA. The peptide corresponds to the novelle carboxyl terminus of a +1 frameshift mutant of amyloid precursor protein (APP^{+1}) and is not present in normal APP (van Leeuwen, F.W. *et al.*, 1998, Science **279**:242). Cysteine was added to the amino terminus for conjugation to affinity matrix. Polyclonal antibody was affinity-purified on a column derivitized with the peptide.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS).

Reconstitution

Reconstitute in 100 µL of PBS containing 0.02% NaN_3.

Storage

Avoid repeated freezing and thawing by aliquoting smaller portions of the reconstituted antibody into Eppendorf tubes and storing at -20° C **in a manual defrost freezer.**

Specificity

The antibody detects human APP^{+1}.

Application

Western blot - An antibody concentration of 0.5 µg/mL is recommended.

Protocols for Immunoblotting

Western blotting

Blotting buffer	Blocking solution	Antibody solution
25 mM Tris, pH 7.5	2% nonfat dry milk in blotting buffer	1% nonfat dry milk in blotting buffer
0.15 M NaCl	pH to 7.5	pH to 7.5
0.05% Tween 20		

1. Transfer the electrophoresed proteins to Immobilon filters (Millipore) and incubate the membrane for 1 hour at room temperature in blocking solution.
2. Incubate the membrane overnight in antibody solution containing 0.5 µg/mL rabbit anti-human APP^{+1}.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of blotting buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature in antibody solution containing a 1:2,000 dilution of HRP-conjugated Protein A (Amersham).
5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
6. Detection was with ECL reagent (Amersham).

Cell lysates for western blottings: To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) at 2 x 10^6 - 1 x 10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

R&D Systems, Inc.
1-800-343-7475

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