NEUROMICS

APP Datasheet

Catalog Number:	RA18003	**Host:**	Rabbit
Product Type:	Affinity purified antibody	**Species Reactivity:**	Rat, Mouse, Human, Monkey
Immunogen Sequence:	Peptide corresponding to residues surrounding Thr668 of human APP695. Antibodies are purified by protein A and peptide affinity chromatography.	**Format:**	Liquid in 10mM sodium HEPES (pH7.5), 150mM NaCl, 100ug BSA and 50% glycerol

Applications: **Western blotting** 1:1000
Immunohistochemistry (paraffin) 1:50
Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.

Storage: Store at -20ºC. Do not aliquot.

Application Notes

APP Antibody detects endogenous levels of several isoforms of both mature and immature amyloid β (A4) precursor protein, including APP695, APP770 and APP751.

Western Blot Protocol

Sample Preparation

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm plate). Immediately scrape cells from plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose (or PVDF) membrane.

Membrane Blocking and Antibody Incubations
Note: Volumes for 10 cm x 10 cm (100 cm^2) membrane; for different sized membranes, adjust vol. accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.
8. Process membranes using enhanced chemiluminescence.

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Solutions and Reagents for Western Blot
Note: Prepare solutions with Milli-Q or equivalently purified water.

1X Phosphate Buffered Saline (PBS)

1X SDS Sample Buffer:
62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red

Transfer Buffer:
25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)

10X Tris Buffered Saline (TBS):
To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).

Nonfat Dry Milk (weight to volume [w/v])

Blocking Buffer:
1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).

Wash Buffer:
1X TBS, 0.1% Tween-20 (TBS/T)

Bovine Serum Albumin (BSA)

Primary Antibody Dilution Buffer:
1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).

Protein A Agarose Beads:
Add 5 ml of 1X PBS to 1.5 g of protein A agarose beads. Shake 2 hours at 4°C; spin down. Wash pellet twice with PBS. Resuspend beads in 1 volume of PBS. (Can be stored for 2 weeks at 4°C.)

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Immunohistochemistry

1. Deparaffinize/hydrate sections:
    a. Incubate sections in three washes of xylene for 5 minutes each.
    b. Incubate sections in two washes of 100% ethanol for 10 minutes each.
    c. Incubate sections in two washes of 95% ethanol for 10 minutes each.
2. Wash sections twice in dH2O for 5 minutes each.
3. Wash sections in PBS for 5 minutes.
4. For antigen unmasking, heat sections in microwave in 10 mM sodium citrate buffer (pH 6.0) for 1 minute at full power followed by 9 minutes at medium power. (Keep slides fully immersed in buffer and maintain temperature at or just below boiling. Exact microwave incubation times need to be determined empirically.) Cool slides for 20 minutes after antigen unmasking.
5. Wash sections in dH2O three times for 5 minutes each.
6. Incubate sections in 1% hydrogen peroxide for 10 minutes.
7. Wash sections in dH2O three times for 5 minutes each.
8. Wash section in PBS for 5 minutes.
9. Block each section with 100–400 µl in dilution buffer for 1 hour at room temperature.
10. Remove solution and add 100–400 µl diluted primary antibody to each section. (Dilute antibody in dilution buffer.) Incubate overnight at 4°C.
11. Remove antibody solution and wash sections in PBS three times for 5 minutes each.
12. Add 100–400 µl secondary antibody, diluted in dilution buffer, to each section. Incubate 30 minutes at room temperature.
13. If using ABC biotin/avidin method, make ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
14. Remove secondary antibody solution and wash sections three times with PBS for 5 minutes each.
15. Add 100–400 µl ABC reagent to each section and incubate for 30 minutes at room temperature.
16. Remove ABC reagent and wash sections three times in PBS for 5 minutes each.
17. Add 100–400 µl DAB reagent to each section and monitor staining closely.
18. As soon as the section turns brown, immerse slides in dH2O.
19. If desired, counterstain sections in hematotoxylin for 10 seconds.
20. Wash sections in dH2O three times for 5 minutes each.
21. Dehydrate sections:
    a. Incubate sections in 95% ethanol two times for 10 seconds each.
    b. Repeat in 100% ethanol, incubating sections two times for 10 seconds each.
    c. Repeat in xylene, incubating sections two times for 10 seconds each.
22. Mount coverslips.

Solutions and Reagents for Immunohistochemistry

Xylene

Ethanol

Distilled H2O (dH2O)

Hematotoxylin

1X Phosphate Buffered Saline (PBS)

10 mM Sodium Citrate Buffer:
To prepare 1 liter, add 2.94 g sodium citrate to 1 liter dH2O. Adjust pH to 6.0.

1% Hydrogen Peroxide:
To prepare, add 10 ml 30% H2O2 to 290 ml dH2O.

Dilution Buffer:
5% normal horse, donkey or goat serum in 0.1% Triton X-100, TBS. Normal serum should be from same species as secondary antibody.

ABC Reagent (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA):
Prepare according to manufacturer's instructions 30 minutes before use.

DAB Reagent:
Add 6.7 µg of 30% hydrogen peroxide to 10 ml dH2O; add this mixture to 10 ml of 1 mg/ml DAB (diaminobenzidine tetrahydrochloride) in PBS, filter.

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