NEUROMICS

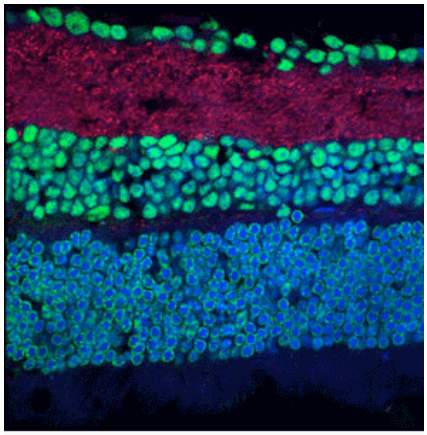
Synapsin Data Sheet

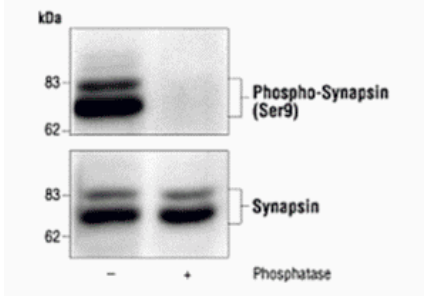
Catalog Number:	RA18010	**Host:**	Rabbit
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Human and Mouse
Immunogen Sequence:	Synthetic peptide corresponding to residues surrounding Ser9 of human synapsin I.	**Format:**	Liquid in 10mM sodium HEPES (pH7.5), 150mM NaCl, 100ug BSA and 50% glycerol.
Applications:	Immunohistochemistry 1:50 (Frozen Sections) Western Blot 1:1000 Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Maintain at +2-8 °C for 3 months or at -20 °C for longer periods. Stable for 1 year. Serum may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20ºC. Repeated freeze/thaw cycles compromise the integrity of the antiserum.		

Application Notes

Description/Data:
The synapsins are a family of proteins that have long been implicated in the regulation of neurotransmitter release at synapses. Specifically, they are thought to be involved in regulating the number of synaptic vesicles available for release via Exocytosis. All synapsins contain a short amino-terminal domain that is highly conserved and phosphorylated by PKA or CaM kinase I. Phosphorylation of synapsin amino-terminal domain at serine 9 inhibits its binding tophospholipids and dissociates synapsins from synaptic vesicles.

Image: Synapsin and CREB staining of of mouse retina. Western blot analysis of mouse brain homogenates, untreated or phosphatase-treated, using Phospho-Synapsin (Ser9) (upper) or Synapsin Antibody (lower).





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Specificity:
Synapsin Antibody detects endogenous levels of total synapsin protein. This antibody is expected to recognize all synapsin isoforms.

Western Blot:
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 cm2) 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.

Immunohistochemistry:
NOTE: All subsequent incubations should be carried out at room temperature, unless otherwise noted, in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading.
1. Block specimen in 5% normal serum from same species as secondary antibody (eg. normal goat serum, normal donkey serum) in PBS/Triton for 60 minutes.
2. While blocking, prepare primary antibody by diluting as indicated on datasheet in PBS/Triton. You will need 50-100 µl per section, 25-50 µl per coverslip, chamber, or well (48 or 96 well plate).
3. Aspirate blocking solution, apply diluted primary antibody.
NOTE: For double-labeling, prepare a cocktail of mouse and rabbit primary antibodies at their appropriate dilutions in PBS/Triton.
4. Incubate overnight at 4°C.
5. Rinse three times in PBS for 5 minutes each.
OPTION: To decrease background stain, rinse in high salt PBS for two minutes between second and third PBS rinses. Be aware, this may reduce specific staining of some antibodies.
NOTE: If using primary antibodies directly conjugated with AlexaFluor® fluorochromes, then skip to step C8.
6. Incubate in fluorochrome-conjugated secondary antibody diluted in PBS/Triton for 1-2 hours at room temperature in dark.
NOTE: For double-labeling, prepare a cocktail of fluorochrome-conjugated anti-mouse and anti-rabbit primary antibodies at their appropriate dilutions in PBS/Triton.
7. Rinse in PBS/high salt PBS as in step 5.
8. Coverslip slides with Vectashield Mounting Medium or apply just enough to cover cells in multiwell plate.
9. Seal slides by painting around edges of coverslips with nail polish.
10. Examine specimens immediately using appropriate excitation wavelength, depending on fluorochrome for best results or store flat at 4°C in dark.

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