

NEUROMICS

phospho-Synapsin (Ser9) Data Sheet

Catalog Number:	RA18009	Host:	Rabbit
Product Type:	Affinity purified antibody	Species Reactivity:	Mouse, Rat 100% homologous with Human
Immunogen Sequence:	Phosphopeptide corresponding to residues surrounding serine 9 of human synapsin I. 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 Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Store at -20°C. Do not aliquot.		

Application Notes

Phospho-Synapsin (Ser9) Antibody detects endogenous levels of synapsin only when phosphorylated at serine 9. The antibody is expected to recognize phosphorylated serine 9 of all synapsin isoforms.

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 μ l per well of 6-well plate or 500 μ l 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 μ l sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 μ l 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.

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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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