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phospho-Mu Opioid Receptor (Ser375) Data Sheet

Catalog Number:	RA18001	**Host:**	Rabbit
Product Type:	Affinity purified antibody	**Species Reactivity:**	Mouse, Human
Immunogen Sequence:	phosphopeptide corresponding to residues surrounding Ser377 of human (homologous to Ser375 of mouse) µ-opioid receptor	**Format:**	Liquid in 10mM sodium HEPES (pH7.5), 150mM NaCl, 100ug BSA and 50% glycerol
Applications:	**Western blotting** 1:1000 **Immunoprecipitation** 1:100 **Immunocytochemistry** 1:100 Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Store at -20ºC. Do not aliquot.		

Application Notes

Phospho-µ-Opioid Receptor (Ser375) Antibody detects overexpressed µ-opioid receptor only when phosphorylated at serine 375 of mouse MOR (or serine 377 of human MOR).

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 the cells off the 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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Immunoprecipiation Protocol

Sample Preparation

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under non-denaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml 1X ice-cold cell lysis buffer plus 1 mM PMSF to each plate (10 cm) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate on ice four times for 5 seconds each.
6. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at –80°C.

Immunoprecipitation

1. Take 200 µl cell lysate and add primary antibody; incubate with gentle rocking overnight at 4°C.
2. Add protein A agarose beads (20 µl of 50% bead slurry). Incubate with gentle rocking for 1–3 hrs at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500 µl of 1X cell lysis buffer. Keep on ice during washes.
4. Resuspend the pellet with 20 µl 3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
5. Heat the sample to 95–100°C for 2–5 minutes.
6. Load the sample (15–30 µl) on SDS-PAGE gel (12–15%).
7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).

Solutions and Reagents for Western Blot and Immunoprecipitaion
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

3X SDS Sample Buffer:
187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

1X Cell Lysis Buffer:
20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM Sodium pyrophosphate, 1 mM b-Glycerolphosphate, 1 mM Na3VO4, 1 µg/ml Leupeptin. *Note: Addition of PMSF before use is suggested.*

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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Immunocytochemistry

Fixation

1. Prepare 4% paraformaldehyde.
2. Treat cells as desired.
3. Wash cells on coverslips once with cool or room temperature PBS
4. Immerse coverslips in 4% paraformaldehyde at room temperature for 30-60 minutes.
5. Wash slips three times for 5 minutes each with 0.1% Triton X-100, TBS at room temperature.

Staining

1. Block all slips with dilution buffer at room temperature for 45–60 minutes. Wash once for 5 minutes with TBS.
2. Dilute the primary antibody as appropriate in dilution buffer. Centrifuging the antibody for 20 minutes at 12,000 x g in a refrigerated microcentrifuge prior to use will remove any aggregated material, thereby reducing background. Apply the diluted antibody to the cells on coverslips and, most critically, incubate **overnight** at 4°C.

   Note: When using any primary or fluorescence-labeled secondary antibody for the first time, titrate out the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.

   Note: You may wish to leave one slip for a secondary antibody only control.
3. Wash all slips three times for 5 minutes each with 0.1% Trition X-100, TBS.
4. Incubate all slips with a dilution of the fluorescence-labeled secondary antibody in dilution buffer for 30–45 minutes at room temperature in the dark.
5. Wash all slips three times for 5 minutes each with 0.1% Trition X-100, TBS in low lighting conditions.
6. Wash all coverslips three times with PBS.
7. Mount coverslips on slides using appropriate anti-fade mounting media.
8. Store slides at room temperature in the dark.

Solutions and Reagents for Immunocytochemistry

Note: Prepare solutions with Milli-Q or equivalently purified water.

1X Phosphate Buffered Saline (PBS)

Fetal Bovine Serum (FBS)

4% Paraformaldehyde:
Prepare day of use either from commercial 16% stock solution or by dissolving paraformaldehyde in 1X PBS by heating at 60°C and stirring until it dissolves. Cool before use.

Tris Buffered Saline (TBS):
50 mM Tris-HCl (pH 7.4), 150 mM NaCl

0.1% Triton X-100:
Prepare stock of 20% Triton in TBS; rotate tube overnight to dissolve. Dilute to 0.1%

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

Coverslip mounting media

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