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ProductInformation

MONOCLONAL ANTI-P35 (CDK5 REGULATOR)
Clones ES19 and ES24
Purified Mouse Immunoglobulin

Product Number **P 0232**

Product Description

Monoclonal Anti-p35 (Cdk5 regulator) (mouse IgG isotype) is derived from the ES19 and ES24 hybridomas produced by the fusion of mouse myeloma cells and splenocytes from a mouse immunized with recombinant human p35. The antibody is purified from ascites fluid using protein G.

Monoclonal Anti-p35 (Cdk5 regulator) specifically recognizes the p35 protein (35 kDa) and cross-reacts with human and bovine. Other species cross-reactivity is unknown. Anti-p35 may be used for immunoprecipitation and is not recommended for immunoblotting.

Cyclin-dependent kinase 5 (Cdk5) and its neuron specific regulator p35 are essential for neuronal migration and for laminar configuration of the cerebral cortex. Cdk5 and p35 tend to concentrate at the leading edges of axonal growth cones and regulates neurite outgrowth in cortical neurons in culture.[1] p35 associates directly with Rac in a GTP dependent manner. Pak1 kinase is also present in the Rac-p35/Cdk5.[1] Increased Cdk5 activity in the brain after ischemia may cause depolymerization of neurocytoskeletons, which can result in neuronal cell death.[2] The association of cell death is unique to Cdk5, since this association is not found with the other Cdks (Cdk 1-8) and cell death.[3]

Reagents

Monoclonal Anti-p35 (Cdk5 regulator) is supplied as purified mouse immunoglobulin in 0.1 M Tris-glycine, pH 7.0, containing 0.15 M NaCl and 0.05% sodium azide.

Protein concentration is approximately 1 mg/ml.

Precautions and Disclaimer

This product contains sodium azide. A material safety data sheet (MSDS) has been sent to the attention of the safety officer at your institution. Consult MSDS for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C –8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Procedures

Immunoprecipitation

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 mg/ml total cell protein in a microcentrifuge tube with PBS (Product No. P3813).
2. Add 4 μg of Monoclonal Anti-p35 to 0.5 – 1 mg cell lysate.
3. Gently rock the reaction mixture at 4 °C overnight.
4. Capture the immunocomplex by adding 100 μl of a washed (in PBS) 1:1 slurry of Protein G-Agarose beads (50 μl packed beads) (Product No. P2294).
5. Gently rock reaction mixture at 4 °C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x *g*), and drain off the supernatant. Wash the beads 3 times with either ice cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50 μl 2X Laemmli sample buffer. The agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. Pellet the beads using a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 μg/ml each aprotinin, leupeptin, pepstatin, 1 mM Na_3VO_4, and 1 mM NaF.

Product Profile
Recommended working concentration is 4 µg to immunoprecipitate p35 from 0.5 mg of a bovine brain lysate.

Note: Not recommended for immunoblotting. In order to obtain best results and assay sensitivities of different techniques and preparations, we recommend determining optimal working dilutions by titration test.

References

1. Nikolic, M., et al., Nature, **395**,194 (1998).
2. Hayashi, T., et al., Neurosci. Lett., **265**, 37 (1999).
3. Ahuja, H. S., et al., Dev. Genet., **21**, 258 (1997).

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