

3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

MONOCLONAL ANTI-v-Src Clone 327 Purified Mouse Immunoglobulin

Product Number S 1686

Product Description

Monoclonal Anti-v-Src (mouse $IgG1\kappa$ isotype) is derived from the hybridoma produced by the fusion of P3X63 Ag8.653 mouse myeloma cells with splenocytes from an immunized BALB/c mouse. Purified Src protein (pp60^{src}) was used as immunogen. ¹ The antibody is purified using Protein A or Protein G.

Monoclonal Anti-v-Src reacts with active Src kinase from human, mouse, and rat by immunoprecipitation, and immunoblotting, ^{1,2} detecting a band of approximately 56 kDa. The antibody is not recommended for immunohistochemistry.

Members of the Src family are intracellular, membraneassociated, non-receptor tyrosine kinases. They transduce signals from transmembrane receptors to intracellular components. Multiple pathways can be stimulated upon Src activation that can lead to a variety of cellular responses including differentiation and proliferation. All Src family members have the same basic structure: an amino terminal Src homology 4 domain (SH4), containing a myristylation signal sequence essential for membrane localization; conserved SH3 and SH2 domains, which mediate protein-protein interactions, separated from the SH4 by a unique region; a catalytic kinase domain in the C-terminal half of the protein; and a short C-terminal tail that negatively regulates the kinase activity.3 The major translation product of the avian Rous sarcoma virus has been designated pp60^{src} and possesses the characteristic protein tyrosine kinase activity. 4,5 The mammalian cellular homolog of pp60^{src} is designated pp60^{c-src}. 6,7 pp60^{c-src} is normally suppressed by phosphorylation at its C-terminus by a kinase, CSK. pp60^{c-src} can be activated by mutation or by various stimuli such as interaction with polyoma virus-encoded middle T antigen.⁸⁻¹⁰ Immunoprecipitates of middle T antigen also contain phosphatidylinositol kinase activity coinciding with the presence of a 81 kDa (p81) protein. 10,111 p81 is phosphorylated by activated pp60 c-src and is thought to be the phosphatidylinositol kinase. 10

Reagents

Monoclonal Anti-v-Src is supplied as 100 μg of purified, lyophilized antibody with 100 μg of bovine serum albumin.

Storage/Stability

Store at 2-8 °C. Do not freeze. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

After reconstitution, store at 2-8 °C with the addition of 15 mM sodium azide, or freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in a "frost-free" freezer is not recommended.

Reconstitution

Resuspend the lyophilized antibody with 1 ml sterile phosphate buffered saline, pH 7.4, or sterile 20 mM Tris containing 0.15 M NaCl, pH 7.4 to yield a final concentration of 100 μ g/ml. Lyophilized antibodies should be resuspended at 4 °C with occasional gentle mixing for at least two hours.

Product Profile

The recommended antibody concentration is 2-5 μ g/ml for immunoblotting using a COLO201 cell extract and chemiluminescence detection.

Note: 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. Lipsich, L. A. et al., J. Virol., 48, 352 (1983).
- Grandori, C., and Hanfusa, H., J. Cell Biol., 107, 2125 (1988).
- 3. Thomas, S. M., and Brugge, J.S., Annu. Rev. Cell Dev. Biol., **13**, 513-609 (1997).
- 4. Wang, L. H., and Hanafusa, H., Virus Res., **9**, 159 (1988).
- Hunter, T., and Sefton, B., Proc. Natl. Sci. USA, 77, 1311 (1980).

- 6. Collett, M. S., et al., Cell, **15**, 1363 (1978).
- Collett, M. S. et al., Proc. Natl. Sci. USA, 76, 3159 (1979).
- 8. Bolen, J. B., et al., Cell, 38, 767 (1984).
- 9. Cartwright, C. A., et al., Mol. Cell Biol., **5**, 2647 (1985).
- Courtneidge, S. A., and Heber, A., Cell, **50**, 1031 (1987).
- 11. Whitman, M., et al., Nature, 315, 239 (1985).

ac/pg 5/00