

Mouse (monoclonal) Anti-Active Src Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number: AHO0051

Lot Number: See product label

Quantity/Volume: $50 \mu g/0.1 \text{ mL}$

Clone Number: 28

Isotype: $IgG_{2a} \kappa \text{ (mouse)}$

Form of Antibody: Purified immunoglobulin in phosphate buffered saline, pH 7.2, with 1% bovine serum

albumin

Preservation: 0.1% sodium azide (Caution: sodium azide is a poisonous and hazardous substance.

Handle with care and dispose of properly.)

Purification: Purified from ascites by affinity chromatography.

Immunogen: KLH conjugated peptide corresponding to amino acid residues 519-536 of human c-Src.

Specificity: This monoclonal antibody recognizes an active form of c-Src which is not phosphorylated

at tyrosine 529. Src (also known as pp60^{src}) is a non-receptor tyrosine kinase involved in signal transduction in many biological systems and implicated in the development of human tumors. There are two critical phosphorylation sites of tyrosine on Src, tyrosine 418 and tyrosine 529 (referring to human Src sequence). The tyrosine 418 is located in the catalytic domain and is one of the autophosphorylation sites. Full catalytic activity of Src requires phosphorylation of tyrosine 418. The tyrosine 529 is located near the carboxyl terminus of Src and acts as a negative regulator, in that Src is held in the inactive form through an intramolecular interaction between the SH2 domain and the carboxyl terminus when tyrosine 529 is phosphorylated by Csk. This conformation blocks phosphorylation of tyrosine 418 at the catalytic domain, thereby preventing Src activation. When tyrosine 529 is dephosphorylated, tyrosine 418 can be maximally phosphorylated and Src becomes active. This antibody also recognizes the other members of Src family including Fyn, Yes,

and Fgr.

Species Reactivity: Human, mouse, and rat. Other species were not tested.

Applications: This antibody is suitable for use in Western blotting, immunocytochemistry and

immunohistochemistry.

Suggested Working

Dilutions:

For Western blotting, the recommended concentration is 1 $\mu g/mL$. The optimal antibody

concentration should be determined for each specific application.

Recommended Positive

Control:

Human MCF-7 cells, mouse 3T3-L1 cells, and rat PC-12 cells.

Storage: Store at 2-8°C. For long term storage, aliquot into small volumes and store at -20°C.

Avoid repeated freeze-thaw cycles to prevent denaturing the antibody.

This product is for research use only. Not for use in diagnostic procedures.

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(Rev 10/08) DCC-08-1089

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| Src (total) ELISA Kit | Cat. # | KHO0161 |
|--|--------|-----------|
| Src [pY418] phosphoELISA TM Kit | Cat. # | KHO0171 |
| Src [pY418] Phosphospecific Antibody | Cat. # | 44-660G |
| Src [pY529] Phosphospecific Antibody | Cat. # | 44-662G |
| Rabbit (polyclonal) anti-Src Antibody | Cat. # | 44-655G |
| Mouse (monoclonal) anti-Src Antibody | Cat. # | AHO1152 |
| c-Src (active) Recombinant Protein | Cat. # | PHO3134/5 |
| Cell Extraction Buffer | Cat. # | FNN0011 |

References:

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Related Products:

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Shanmugam, M., et al. (1998) Association of PKC delta and active Src in PMA-treated MCF-7 human breast cancer cells. Oncogene 16(13):1649-1654.

Kovacic, B., et al. (1998) c-Src activation plays a role in endothelin-dependent hypertrophy of the cardiac myocyte. J. Biol. Chem. 273(52):35185-35193.

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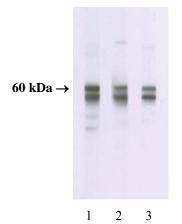
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Yamamoto, Y., et al. (2002) Expression and subcellular distribution of the active form of c-Src tyrosine kinase in differentiating human endometrial stromal cells. Mol. Hum. Reprod. 8(12):1117-1124.

Ghalayini, A.J., et al. (2002) Light-dependent association of Src with photoreceptor rod outer segment membrane proteins in vivo. J. Biol. Chem. 277(2):1469-1476.

Zhao, Y.L., et al. (2003) Active Src expression is induced after rat peripheral nerve injury. Glia. 42(2):184-193.

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Western Blot Analysis

Proteins from cell extracts of human MCF-7 cells (lane 1), mouse 3T3-L1 cells (lane 2), and rat PC-12 cells (lane 3) were resolved by SDS-PAGE and transferred to PVDF. The membranes were incubated with this active-Src monoclonal antibody (clone 28) at a concentration of 1 μg/mL for two hours at room temperature. After washing, the membranes were incubated with a goat F(ab')₂ anti-mouse IgG alkaline phosphatase conjugated antibody (Cat. # AMI4405) at a 1:2000 dilution. Bands were detected with CDP-substrate using the WesternStarTM method (Tropix) and Kodak BioMax film.

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