Technical Data Sheet

Alexa Fluor® 488 Mouse anti-Src (pY418)

Product Information

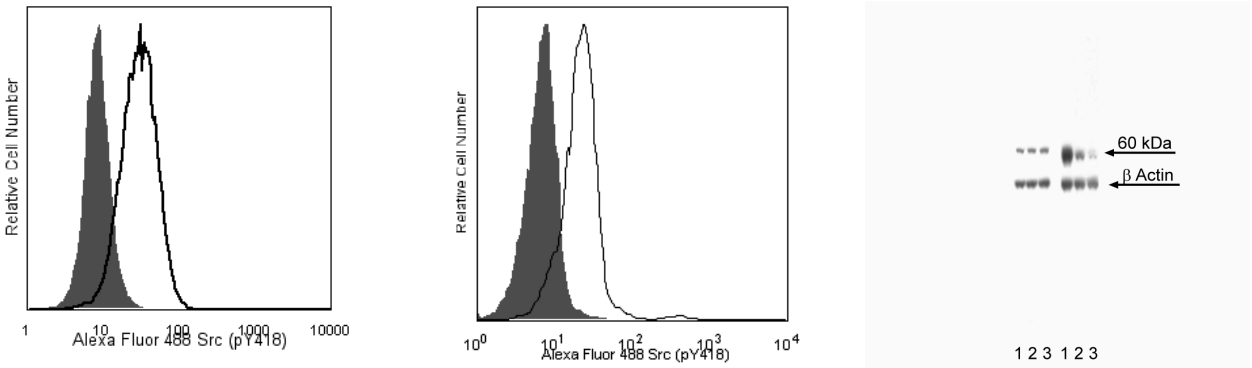
Material Number:	**560095**
Alternate Name:	c-Src, p60-Src, SRC, SRC1
Size:	50 tests
Vol. per Test:	20 µl
Clone:	K98-37
Immunogen:	Phosphorylated Human Src Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	Confirmed: Human Predicted: Chicken, Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The non-receptor protein tyrosine kinases of the Src family have similar domain structures with considerable sequence homologies. They are associated with cell membranes and are activated by a wide variety of cell-surface receptors. Through their participation in cell-signaling cascades that mediate an array of cellular responses, they control essential cellular activities.

Src, the prototype of the Src family, is encoded by the *SRC* protooncogene, which is a homolog of the *v-Src* gene of the Rous sarcoma virus. Src's oncogenic potential may be a consequence of its role as a regulator of cell growth, morphology, motility, and adhesion. The protein contains domains that mediate protein-protein interactions and regulation of its tyrosine kinase activity. Mechanisms that regulate Src activity include phosphorylations of serines and tyrosines in most of the domains and interactions with receptor proteins, integrins, and other binding proteins. In particular, autophosphorylation at tyrosine 418 (Y418) activates tyrosine kinase activity. Phosphorylations at other sites are mediated by kinases such as cdc2, CSK, and CHK in signaling cascades and may up- or down-regulate Src activity.

The K98-37 monoclonal antibody recognizes the phosphorylated Y418 in the protein kinase domain of activated Src. The orthologous phosphorylation sites in chicken, mouse, and rat Src are Y415, Y423, and Y418, respectively. Because of sequence similarities in this region, it may also recognize the homologous sites of some other Src family members: Lyn (pY396), Fyn (pY420), Hck (pY410), Lck (pY394), and Yes (pY425).



Analysis of Src (pY418) in human T leukemia cells and peripheral blood lymphocytes. *Jurkat cells (ATCC TIB152, left panel) and human peripheral blood mononuclear cells (PBMC, middle panel) were either treated with 1µM Staurosporine (EMD Biosciences, Cat. No. 569397) for 2 hours at 37ºC (shaded histogram) or untreated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37ºC, permeabilized with BD™ Phosflow Perm Buffer III (Cat. No. 558050) on ice for 30 minutes, and then stained with Alexa Fluor® 488 Mouse anti-Src (pY418). Lymphocytes were selected by their scatter profile during analysis of the PBMC. The data demonstrates that the level of phosphorylation of Src (pY418) decreases when protein kinase activity is inhibited by the treatment. Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.*

The specificity of mAb K98-37 was confirmed by western blot analysis (right panel) using unconjugated Rabbit anti-Src (Cell Signaling Technology, Cat. No. 2123, left blot) and unconjugated Mouse anti-Src (pY418) (right blot) monoclonal antibodies on lysates of Jurkat cells that were untreated (lanes 1) or treated with Staurosporine for 1 hour (lanes 2) or 2 hours (lanes 3). Src (pY418) is identified as a band of 60 kDa that is reduced by Staurosporine treatment.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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The purified or conjugated mAb was characterized by flow cytometry (Flow) and western blot (WB) using these model systems:

Method	*Species*	*Cells*	*Treatment*	*Fixation*	*Perm buffer*	*Result*
Flow	Human	A-431	none	Cytofix	Perm III	Positive expression
	Human	A-431	Phospho peptide	Cytofix	Perm III	Blocking
	Human	A-431	Non-phospho peptide	Cytofix	Perm III	No blocking
	Human	Jurkat	Staurosporine	Cytofix	Perm III	Down-regulation
	Human	PBMC	Staurosporine	Cytofix	Perm I, II, or III	Down-regulation
WB	Human	A-431	Phospho peptide			Blocking of 60-kDa band
	Human	A-431	Non-phospho peptide			No blocking of 60-kDa band
	Human	Jurkat	Staurosporine			60-kDa band down-regulated
	Human	PBMC	Staurosporine			60-kDa band down-regulated

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Either BD Cytofix™ fixation buffer or BD™ Phosflow Fix Buffer I may be used for cell fixation. Any of the three BD™ Phosflow permeabilization buffers may be used.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
557870	Fix Buffer I	250 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
3. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

Alvarez RH, Kantarjian HM, Cortes JE. The role of Src in solid and hematologic malignancies. Development of new-generation Src inhibitors. *Cancer.* 2006; 107(8):1918-1929. (Biology)

Arias-Salgado EG, Lizano S, Sarkar S, Brugge JS, Ginsberg MH, Shattil SJ. Src kinase activation by direct interaction with the integrin β cytoplasmic domain. *Proc Natl Acad Sci U S A.* 2003; 100(23):13298-13302. (Biology)

Frame MC. Newest findings on the oldest oncogene; how activated src does it. *J Cell Sci.* 2004; 117(7):989-998. (Biology)