Technical Data Sheet

Alexa Fluor® 488 Mouse anti-Syk (pY348)

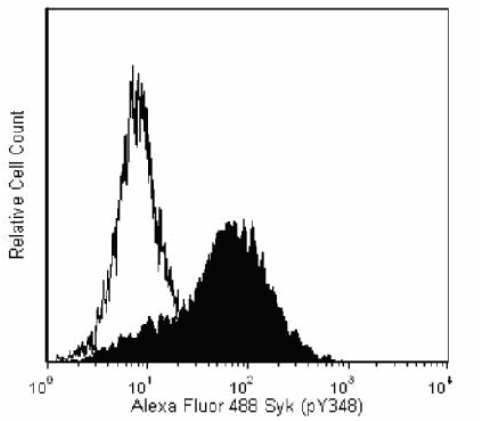
Product Information

Material Number:	**560081**
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	I120-722
Immunogen:	Phosphorylated Human Syk Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

Syk is a non-receptor protein-tyrosine kinase that is closely related to ZAP70 and plays crucial roles in the development and receptor-mediated signaling of most leukocytes and in vascular integrity. Syk is expressed in hematopoietic cells, including B lymphocytes, immature (CD4, CD8 double-negative and double-positive) thymocytes, and myeloid cells, epithelial cell lines, and normal breast tissue. Mature (CD4 or CD8 single-positive) thymocytes and peripheral αβ TCR-bearing T lymphocytes have very low or undetectable levels of Syk. Syk contributes to the signal transduction process by binding to ITAMs (*I*mmunoreceptor *T*yrosine-based *A*ctivation *M*otifs) of immune receptors, including Igα and Igβ (CD79a and b), TCRζ, CD3ε, and FcRγ. Upon receptor activation, Syk binds to phosphorylated ITAMs via its two N-terminal SH2 domains thereby activating Syk and causing tyrosines in the interdomain, between the SH2 and Kinase domains of Syk, to undergo auto-phosphorylation and phosphorylation by Lyn. The tyrosine 348 phosphorylation site (pY348) in human Syk is orthologous to tyrosine 342 in mouse and rat Syk and tyrosine 315 in human ZAP70. This phosphorylated site can act as a binding site for other signaling molecules, such as PLCγ, Vav, and Fgr.

The I120-772 antibody is specific for human Syk (pY348) and does not cross-react with phosphorylated Zap70. The orthologous phosphorylation site in mouse and rat Syk is Y342.



Flow cytometric analysis for Syk (pY348). *Jurkat cells (Human T-cell leukemia; ATCC TIB-152) were serum starved overnight, then either treated with 5 mM H2O2 at 37°C for 15 minutes (shaded histogram) or were untreated (open histogram). The cells were harvested and fixed with pre-warmed BD Cytofix™ Fixation Buffer (Cat. No. 554655) for 10 minutes at 37°C, permeabilized with BD Phosflow™ Perm buffer II (Cat. No. 558052) on ice for 30 minutes, and then stained with Alexa Fluor® 488 Mouse anti-Syk (pY348). The histograms were derived from the light scattering characteristics of the viable cells. The data demonstrates the upregulated phosphorylation of the treated cells versus untreated cells. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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.

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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	PBMC	Surface IgM crosslinking	Lyse/Fix or Fix I	Perm I, II, or III	Up-regulated expression on B lymphocytes
		Ramos	Surface IgM crosslinking	Lyse/Fix	Perm II	Up-regulated expression
		Jurkat	H2O2	Cytofix	Perm II	Up-regulated expression
	Mouse	Splenocytes	Surface IgM crosslinking	Lyse/Fix	Perm II	Up-regulated expression on B lymphocytes
WB	Human	Ramos	Pervanadate			72 kDa band induced
			Pervanadate + phosphor peptide			Blocking of 72 kDa band
			Pervanadate + unrelated phospho peptide			No blocking

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human lymphoid cell lines, peripheral blood mononuclear cells, and mouse splenocytes using BD Phosflow™ Fix Buffer I or Lyse/Fix Buffer. Any of the three BD Phosflow™ permeabilization buffers may be used.

Suggested Companion Products

Catalog Number	Name	Size	Clone
557870	Fix Buffer I	250 mL	(none)
558049	Lyse/Fix Buffer 5X	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)
554655	Fixation Buffer	100 mL	(none)

Product Notices

1. 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).
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. 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.
4. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
7. 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.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

References

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Latour S, Veillette A. Proximal protein tyrosine kinases in immunoreceptor signaling. *Curr Opin Immunol.* 2001; 13:299-306. (Biology)

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Zhang J, Benestein E, Siraganian RP. Phosphorylation of Tyr342 in the linker region of Syk is critical for FceRI signaling in mast cells. *Mol Cell Biol.* 2002; 22:8144-8154. (Biology)

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