Technical Data Sheet

Alexa Fluor® 647 Mouse anti-CrkL (pY207)

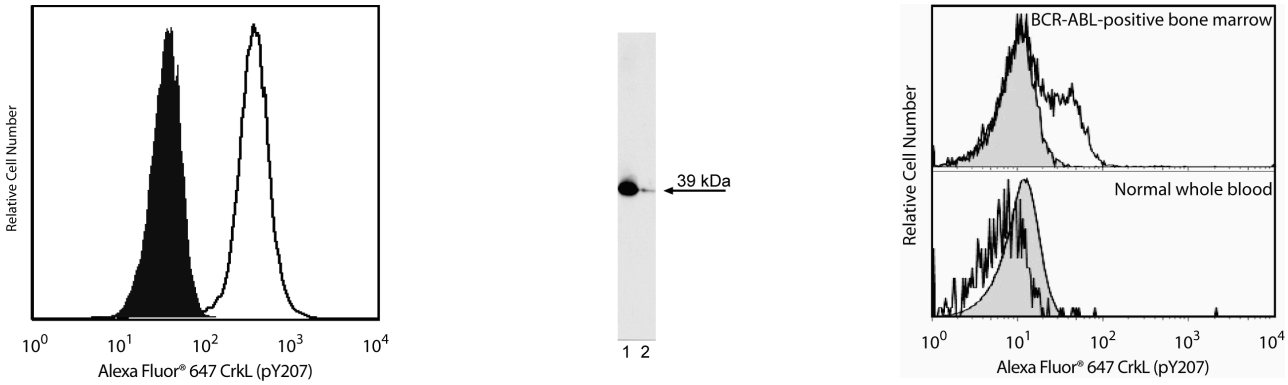
Product Information

Material Number:	**560790**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	K30-391.50.80
Immunogen:	Phosphorylated Human, Mouse, and Rat CrkL Peptide
Isotype:	Mouse (BALB/c) IgG2a, κ
Reactivity:	QC Testing: Human Predicted due to immunogen sequence identity: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

*Crk-L*ike (CrkL) is an adaptor protein that is preferentially expressed in hematopoietic cells and is encoded by a gene that is homologous to the viral oncogene *v-crk* (*c*hicken tumor virus no. 10 *r*egulator of *k*inase). Its SH2 and SH3 domains bind to a variety of effector proteins, such as paxillin, p130Cas, c-Cbl, c-Abl, and C3G. These interactions are involved in the regulation of cellular migration, adhesion, and transformation. Tyrosine 207 (Y207) of CrkL is phosphorylated in hematopoietic cells that express the BCR-ABL fusion protein. This site may be a negative regulator of protein complex formation and biological activity.

The K30-391.50.80 monoclonal antibody recognizes the phosphorylated Y207 of human CrkL.



Analysis of CrkL (pY207) in human chronic myelogenous leukemia cell line and BCR-ABL expressing cells.
LEFT: K562 cells (ATCC CCL-243) were either treated with the tyrosine kinase inhibitor, imatinib, at 25 µM (LC Laboratories, shaded histogram) for 2 hours at 37°C or untreated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37°C, then permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-CrkL (pY207). Flow cytometry was performed on a BD™ FACSCanto II flow cytometry system.
MIDDLE: The specificity of mAb K30-391.50.80 was confirmed by western blot using unconjugated antibody on lysates from control (lane 1) and imatinib-treated (lane 2) K562 cells. CrkL (pY207) is identified as a band of 39 kDa that has decreased intensity in the treated cells.
RIGHT: A BCR-ABL expressing human bone marrow sample (top profile) and normal human whole blood were lysed and fixed (BD™ Phosflow Lyse/Fix Buffer, Cat. No. 558049) for 10-15 minutes at 37°C and then permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes. The cells were stained with PE Mouse Anti-Human CD34 (Cat. No. 550761), PerCP Mouse Anti-Human CD3 (Cat. No. 347344), and Alexa Fluor® 647 Mouse anti-CrkL (pY207). In each profile, the filled histogram represents CD3-positive cells and the open histogram represents CD34-positive cells. Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 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*	*mAb format*	*Result*
Flow	Human	Normal whole blood*	None	Lyse/Fix	Perm III, IV	Alexa Fluor® 647	Not detected†
	Human	BCR-ABL-positive whole blood, PBMC, bone marrow*	None	Lyse/Fix	Perm III, IV	Alexa Fluor® 647	Some expression on CD34-positive cells†
	Human	BCR-ABL-positive whole blood, bone marrow*	imatinib or dasatinib‡	Lyse/Fix	Perm III, IV	Alexa Fluor® 647	Not detected or very weak expression on CD34-positive cells
	Human	K562	None	Cytofix	Perm III, IV	purified, all conjugates	Positive
	Human	K562	imatinib‡‡	Cytofix	Perm III, IV	purified, all conjugates	Decreased
WB	Human	K562	None			purified	39-kDa band observed
	Human	K562	imatinib‡‡			purified	39-kDa band decreased

* Fresh samples (frozen or ≤24 hours old), †see example in data sheet for Cat. No. 560790, ‡ in vivo, ‡‡ in vitro

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human whole blood, peripheral blood mononuclear cells (PBMC), and bone marrow (using BD Phosflow Lyse/Fix Buffer and Perm Buffer III or IV) and cell lines (using BD Cytofix™ Fixation Buffer and BD Phosflow Perm Buffer III or IV).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
560746	Perm Buffer IV 10×	50 ml	(none)
554656	Stain Buffer (FBS)	500 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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
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

Arai A, Nosaka Y, Kohsaka H, Miyasaka N, Miura O. CrkL activates integrin-mediated hematopoietic cell adhesion through the guanine nucleotide exchange factor C3G. *Blood.* 1999; 93(11):3713-3722. (Biology)

Feller SM. Crk family adaptors-signalling complex formation and biological roles. *Oncogene.* 2001; 20:6348-6371. (Biology)

Senechal K, Heaney C, Druker B, Sawyers CL. Structural requirements for function of the Crkl adapter protein in fibroblasts and hematopoietic cells. *Mol Cell Biol.* 1998; 18(9):5082-5090. (Biology)