

## Technical Data Sheet

## PE Mouse anti-CrkL (pY207)

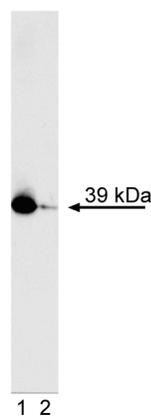
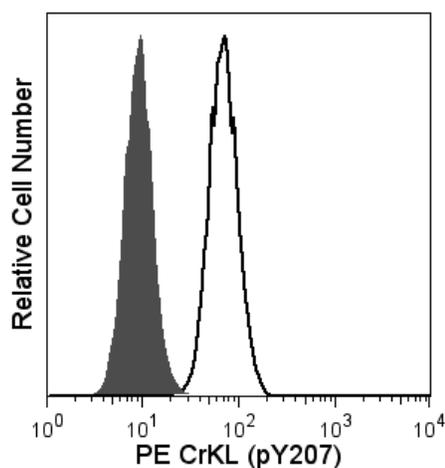
## Product Information

Material Number:	560788
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 Reactivity: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

## Description

Crk-Like (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* (chicken tumor virus no. 10 regulator of kinase). 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.** 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, left figure). The cells were fixed (BD Cytotix™ 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 PE Mouse anti-CrkL (pY207, Cat. No. 560788). Flow cytometry was performed on a BD FACSCanto™ II flow cytometry system. The specificity of mAb K30-391.50.80 was confirmed by western blot (right figure) 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.

## 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 with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

## Application Notes

## Application

Intracellular staining (flow cytometry)

Routinely Tested

## Recommended Assay Procedure:

This PE-conjugated antibody is suitable for intracellular staining of cell lines using BD Cytotix™ Fixation Buffer and BD Phosflow™ Perm Buffer III or IV. We have confirmed that the Alexa Fluor® 647 conjugate (Cat. No. 560790) is suitable for staining leukocytes from human peripheral blood and bone marrow.

## BD Biosciences

bdbiosciences.com

United States 877.232.8995 Canada 866.979.9408 Europe 32.2.400.98.95 Japan 0120.8555.90 Asia Pacific 65.6861.0633 Latin America/Caribbean 55.11.5185.9995

For country contact information, visit [bdbiosciences.com/contact](http://bdbiosciences.com/contact)

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton, Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

© 2016 BD. BD, the BD Logo and all other trademarks are property of Becton, Dickinson and Company.

560788 Rev. 2



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 <sup>†</sup>
	Human	BCR-ABL-positive whole blood, PBMC, bone marrow*	None	Lyse/Fix	Perm III, IV	Alexa Fluor® 647	Some expression on CD34-positive cells <sup>†</sup>
	Human	BCR-ABL-positive whole blood, bone marrow*	imatinib or dasatinib <sup>‡</sup>	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 <sup>‡‡</sup>	Cytofix	Perm III, IV	purified, all conjugates	Decreased
WB	Human	K562	None			purified	39-kDa band observed
	Human	K562	imatinib <sup>‡‡</sup>			purified	39-kDa band decreased

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

## Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 mL	(none)
558050	Perm Buffer III	125 mL	(none)
560746	Perm Buffer IV 10×	50 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)

## Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100- $\mu$ l experimental sample (a test).
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. 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.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
5. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.

## 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)

## BD Biosciences

[bdbiosciences.com](http://bdbiosciences.com)

United States 877.232.8995 Canada 866.979.9408 Europe 32.2.400.98.95 Japan 0120.8555.90 Asia Pacific 65.6861.0633 Latin America/Caribbean 55.11.5185.9995

For country contact information, visit [bdbiosciences.com/contact](http://bdbiosciences.com/contact)

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton, Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

© 2016 BD. BD, the BD Logo and all other trademarks are property of Becton, Dickinson and Company.

560788 Rev. 2

