Technical Data Sheet

Alexa Fluor® 647 Mouse anti-p130Cas (pY249)

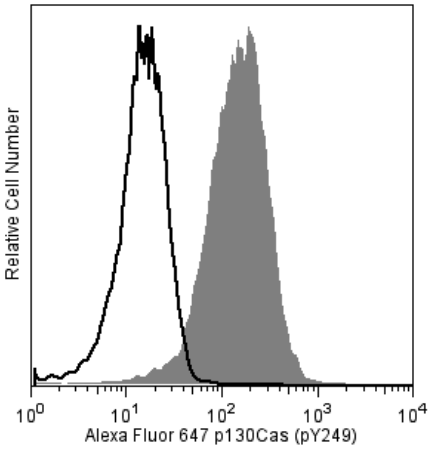
Product Information

Material Number:	**558547**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	J169-757.12.2
Immunogen:	Phosphorylated Human p130Cas
Isotype:	Mouse IgG2b, κ
Reactivity:	Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

p47v-crk (v-Crk) is the product of a transforming gene, v-crk, that was isolated from avian sarcoma viruses. The v-Crk protein is a fusion product of viral Gag protein and a part of cellular Crk that includes SH2 and SH3 domains. v-Crk-induced transformation increases tyrosine phosphorylation of several cellular proteins, including p130Cas (*C*RK-*a*ssociated *s*ubstrate). The p130Cas is tightly associated with v-Crk via the SH2 domain of v-Crk. Tyrosine phosphorylation of p130Cas occurs in conjunction with cellular transformation in cells that express v-Src or v-Crk. This phosphorylation leads to a change in p130Cas localization from the cytoplasm to the cell membrane and, possibly, to the nucleus. Since p130Cas also associates with v-Src, it may be a v-Src substrate. Several phosphorylation sites have been described in p130Cas upon Fibroblast Growth Factor stimulation, and phosphorylated tyrosine (Y249) might function as a binding site for the Crk-adaptor molecule.

The J169-757.12.2 monoclonal antibody recognizes the phosphorylated Y249 of human p130Cas. The orthologous phosphorylation sites in mouse and rat p130Cas are Y253 and Y347, respectively.



Analysis of p130Cas (pY249) in activated human Burkitt's lymphoma. *Ramos cells were serum starved overnight and then either stimulated with 5 mM hydrogen peroxide for 15 minutes (shaded histogram) or unstimulated (open histogram). The cells were fixed (BD™ Phosflow Fix Buffer I, Cat. No. 557870) 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-p130Cas (pY249). 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.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
558050	Perm Buffer III	125 ml	(none)
557870	Fix Buffer I	250 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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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. 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® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
5. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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

Goldberg GS, Alexander DB, Pellicena P, Zhang Z-Y, Tsuda H, Miller WT. Src phosphorylates Cas on tyrosine 253 to promote migration of transformed cells. *J Biol Chem.* 2003; 278(47):46533-46540.(Biology)

Hinsby AM, Olsen JV, Bennett KL, Mann M. Signaling initiated by overexpression of the fibroblast growth factor receptor-1 investigated by mass spectrometry. *Mol Cell Proteomics.* 2003; 2(1):29-36.(Biology)