Technical Data Sheet

Alexa Fluor® 647 Mouse anti-Pyk2 (pY402)

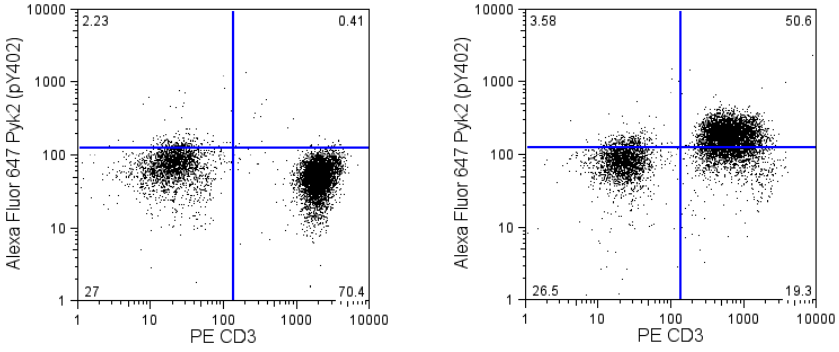
Product Information

Material Number:	**560256**
Alternate Name:	Protein tyrosine kinase 2β, FAK2, FADK 2, CAKβ, CADTK, RAFTK
Size:	50 tests
Vol. per Test:	20 µl
Clone:	L68-1256.272
Immunogen:	Phosphorylated Human Pyk2 Peptide
Isotype:	Mouse (BALB/c) IgG2b, κ
Reactivity:	QC Testing: Human Predicted: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

*P*roline-rich *t*yrosine *k*inase *2* (Pyk2) is a member of the FAK subfamily of tyrosine protein kinases. Pyk2 responds to a wide variety of inflammatory and stress stimuli, such as cytokines, ligation of integrins or antigen receptors, mechanical strain, and cellular depolarization. A common characteristic of most Pyk2 stimuli is an increase in cytosolic free calcium. Pyk2 is involved in signaling pathways that regulate vital cellular functions in hematopoietic, epithelial, neuronal, endothelial, and other cell types. Activation of Pyk2 initiates its autophosphorylation at tyrosine 402 (Y402), followed by Src-mediated phosphorylation of other Pyk2 tyrosine sites, which enhances its activity and leads to downstream signal transduction through the MAPK and JNK pathways.

The L68-1256.272 monoclonal antibody recognizes the phosphorylated Y402 of activated Pyk2.



Analysis of Pyk2 (pY402) in human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells (PBMC) were either untreated (left panel) or stimulated by cross-linking of CD3 and CD28 with NA/LE Mouse anti-Human CD3 mAb UCHT1 (Cat. No. 555329) and NA/LE Mouse anti-Human CD28 mAb CD28.2 (Cat. No. 555725) on ice for 15 minutes followed by Purified Goat anti-Mouse Ig (Cat. No. 553998) on ice for 15 minutes, and then allowed to undergo phosphorylation at 37°C for 5 minutes (right panel). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37°C, permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, blocked with normal mouse immunoglobulin, and then stained with Alexa Fluor® 647 Mouse anti-Pyk2 (pY402) and PE Mouse anti-human CD3 mAb SK7 (Cat. No. 347347). For data analysis, lymphocytes were selected by their scatter profile. The data demonstrates that the upregulated phosphorylation of Pyk2 was restricted to the CD3-positive T lymphocytes under these stimulation conditions. Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.*

The specificity of mAb L68-1256.272 was confirmed by western blot analysis using unconjugated antibody on lysates from PBMC that were untreated (lane 1) or stimulated by cross-linking of CD3 and CD28 (lane 2). Pyk2 (pY402) is identified as a band of 116 kDa, with increased intensity in the stimulated cells.

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*	*Result*
Flow	Human	Jurkat	Hydrogen peroxide	Cytofix or Fix I	Perm III	Induction observed
	Human	PBMC	CD3/CD28 crosslinking	Cytofix or Fix I	Perm I, II, or III	Induction observed
WB	Human	Jurkat	Hydrogen peroxide			116-kDa band induced
	Human	Jurkat	anti-CD3			Unsatisfactory
	Human	PBMC	CD3/CD28 crosslinking			116-kDa band induced

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. 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. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
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

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Gluck SL. Acid sensing in renal epithelial cells. *J Clin Invest.* 2004; 114(12):1696-1699. (Biology)

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