Technical Data Sheet

Alexa Fluor® 647 Mouse anti-PLC-γ2

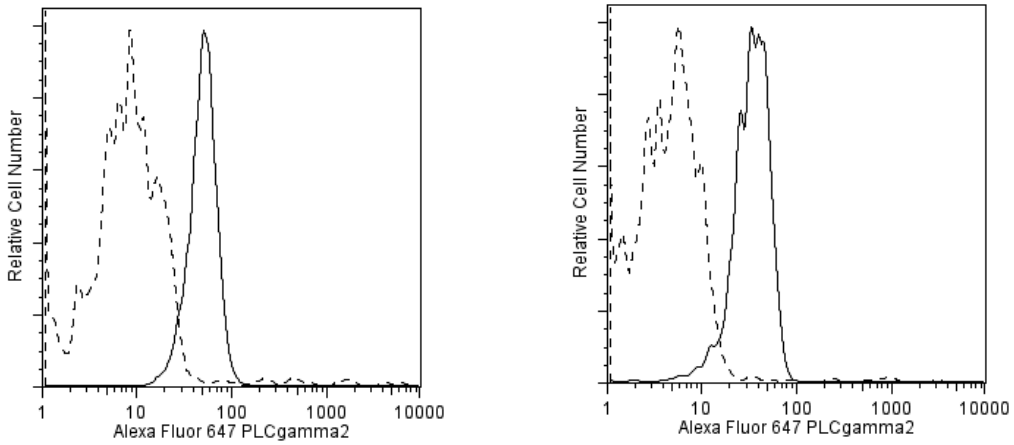
Product Information

Material Number:	**560136**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	K86-1161
Immunogen:	Phosphorylated Human PLC-γ2 Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Confirmed in Development: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The Phospholipase C (PLC) isozymes hydrolyze phosphatidyl inositol biphosphate to inositol triphosphate and diacylglycerol. The former causes release of calcium from the endoplasmic reticulum, while the latter is an activator of Protein Kinase C. Within the PLC family, PLC-γ is the only member that contains SH2 and SH3 domains. These domains enable it to interact with receptor tyrosine kinases and become enzymatically activated via phosphorylation. It exists as two isoforms: 1) PLC-γ1, which is ubiquitously expressed, and 2) PLCγ2, found primarily in the lymphoid system. PLC-γ is essential for growth factor-induced cell motility and mitogenesis. Overexpression of PLC-γ is evident in several forms of cancer, and it has been identified as a key mediator of PDGF-dependent cellular transformation. Thus regulation of PLC-γ activity by growth factors is involved in cell growth and transformation.

Although the immunogen for generation of the K86-1161 monoclonal antibody was a phosphorylated peptide, peptide blocking studies demonstrated that the mAb recognizes PLC-γ2 regardless of phosphorylation status. This antibody was raised to a unique region of PLC-γ2 and is predicted not to crossreact with PLC-γ1



Analysis of PLC-γ2 in human peripheral blood lymphocytes. *Human whole blood was lysed and fixed with 1X BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) for 10-15 minutes at 37ºC, then permeabilized (BD Phosflow™ Perm Buffer II, Cat. No. 558052) on ice for 30 minutes, and then stained with Alexa Fluor® 488 Mouse Anti-Human CD3 mAb UCHT1 (Cat. No. 557694), PerCP-Cy™5.5 anti-human CD20 mAb H1(FB1) (Cat. No. 558021), and either Alexa Fluor® 647 Mouse anti-PLC-γ2 (solid-line histograms) or Alexa Fluor® 647 Mouse IgG1, κ Isotype control mAb MOPC-21 (Cat. No. 557783, dashed-line histograms). The figures show lymphocyte subpopulations that were selected by their scatter profile and surface antigen expression. PLC-γ2 expression on CD20-positive CD3-negative B lymphocytes (left panel) and CD20-negative CD3-negative NK cells (right panel) are displayed. There was little detectable expression of PLC-γ2 in the CD20-negative CD3-positive T lymphocytes (not shown). 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® 647 under optimum conditions, and unreacted Alexa Fluor® 647 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	Ramos	none	Cytofix	Perm III	positive expression
	Human	Ramos	Phospho peptide	Cytofix	Perm III	Blocking
	Human	Ramos	non-phospho peptide	Cytofix	Perm III	Blocking
	Human	Whole blood	none	Lyse/Fix	Perm II	B lymphocytes & NK cells positive
	Mouse	Splenocytes	none	Lyse/Fix	Perm II	B lymphocytes positive
	Mouse	Thymocytes	none	Lyse/Fix	Perm II	negative
WB	Human	Ramos	none			150 kDa

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This PLC-γ2-specific antibody conjugate may be used with conjugates of anti-PLC-γ2 (pY759) mAb K86-689.37 to distinguish the expression of total versus phosphorylated PLC-γ2.

This antibody conjugate is suitable for intracellular staining of human whole blood and mouse splenocytes using the BD Phosflow™ Lyse/Fix Buffer and the BD Phosflow™ Perm Buffer II.

Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558052	Perm Buffer II	125 ml	(none)
557783	Alexa Fluor® 647 Mouse IgG1 κ Isotype control	50 tests	MOPC-21

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. An isotype control should be used at the same concentration as the antibody of interest.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. 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.
8. Cy is a trademark of Amersham Biosciences Limited.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
10. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Kim YJ, Sekiya F, Poulin B, Bae YS, Rhee SG. Mechanism of B-cell receptor-induced phosphorylation and activation of phospholipase C-γ2. *Mol Cell Biol.* 2004; 24(22):9986-9999. (Biology)

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Ozdener F, Dangelmaier C, Ashby B, Kunapuli SP, Daniel JL. Activation of phospholipase Cγ2 by tyrosine phosphorylation.. *Mol Pharmacol.* 2002; 62(3):672-679. (Biology)

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