Technical Data Sheet

Alexa Fluor® 647 Mouse anti-PKA RIIα (pS99)

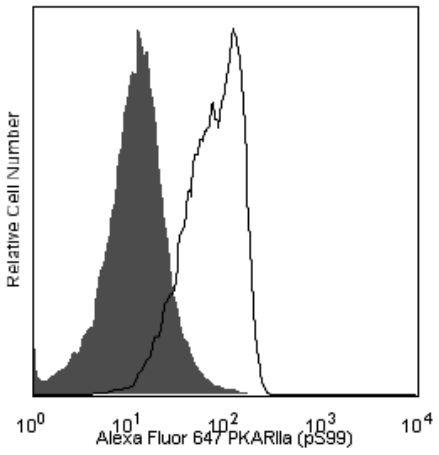
Product Information

Material Number:	**560164**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	I65-856.286
Immunogen:	Phosphorylated Human PKA RIIα Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

c**A**MP-dependent **P**rotein **K**inase (PKA) is composed of two distinct subunits: catalytic (C) and regulatory (R). Four regulatory subunits have been identified: RIα, RIβ, RIIα, and RIIβ. These subunits define type I and II PKAs. Following binding of cAMP, the regulatory subunits dissociate from the catalytic subunits, rendering the enzyme active. Type I and II holoenzymes have three potential C subunits (Cα, Cβ, or Cγ). Type II PKA can be distinguished by autophosphorylation of the R subunits, while type I PKA binds Mg/ATP with high affinity. The cAMP-dependent autophosphorylation of the human RIIα subunit occurs at Serine 99 (S99) [Entrez Protein Accession #CAA33094]. Most cells express both type I and type II PKAs. Although the Rα isoforms are ubiquitously expressed, the Rβ isoforms are predominantly found in nervous and adipose tissues. In addition to their enzyme regulatory activity, the RIIα and RIIβ subunits determine the subcellular location of the holoenzymes via their interactions with specific intracellular anchoring proteins.

The I65-856.286 antibody recognizes human PKA RIIα phosphorylated at S99.



Analysis of PKA RIIα (pS99) in human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells (PBMC) were either treated with 1µM Staurosporine (EMD Biosciences, Cat. No. 569397) for 2hours at 37ºC (shaded histogram) or untreated (open histogram). The PBMC were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37ºC, permeabilized with BD™ Phosflow Perm Buffer III (Cat. No. 558050) on ice for 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-PKA RIIα (pS99). For data analysis, lymphocytes were selected by their scatter profile. The data demonstrates that the level of phosphorylation of PKA RIIα decreases when protein kinase activity is inhibited by the treatment. 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.

BD Biosciences

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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	Daudi	Staurosporine	Cytofix	Perm III	Unsatisfactory
	Human	EA-hy 926	Staurosporine	Cytofix	Perm III	Down-regulation
	Human	HeLa S3	Calyculin A + Okadaic Acid	Cytofix	Perm III	Unsatisfactory
	Human	Jurkat	Staurosporine	Cytofix	Perm III	Down-regulation
	Human	PBMC	Staurosporine	Cytofix	Perm I, II, or III	Down-regulation
WB	Human	Daudi	Staurosporine			no band
	Human	EA-hy 926	Staurosporine			51-kDa band down-regulated
	Human	EA-hy 926	Calyculin A + Okadaic Acid			no induction
	Human	HeLa S3	Calyculin A + Okadaic Acid			51-kDa band induced
	Human	Jurkat	Staurosporine			51-kDa band down-regulated
	Human	PBMC	Staurosporine			51-kDa band down-regulated

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)
558050	Perm Buffer III	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)

Product Notices

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

Francis SH, Corbin JD. Structure and function of cyclic nucleotide-dependent protein kinases. *Annu Rev Physiol.* 1994; 56:237-272. (Biology)

Scott JD, Stofko RE, McDonald JR, Comer JD, Vitalis EA, Magili JA. Type II regulatory subunit dimerization determines the subcellular localization of the cAMP-dependent protein kinase. *J Biol Chem.* 1990; 35:21561-21566. (Biology)