Technical Data Sheet

Alexa Fluor® 488 Mouse anti-PKA[RIIβ] (pS114)

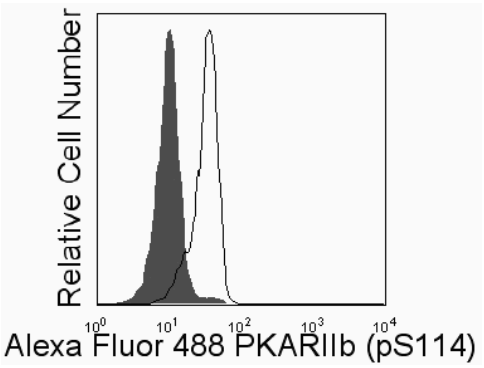
Product Information

Material Number:	**560204**
Alternate Name:	KAP3, PKAR2B, PRKAR2B
Size:	50 tests
Vol. per Test:	20 µl
Clone:	47/PKA
Immunogen:	Phosphorylated Human PKA[RIIβ] peptide Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	Confirmed by flow cytometry: Human Confirmed by western blot using purified antibody (Cat. No. 612550): Human, Rat Predicted: Mouse
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 type II holoenzymes have three potential C subunits (Cα, Cβ, or Cγ). Most cells, including T lymphocytes, express both type I and type II PKAs. RIIα expression is associated with cellular transformation, while RIIβ expression correlates with mitotic arrest and cellular differentiation. 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 RIIβ subunits occurs at serine 114 (S114). 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 47/PKA monoclonal antibody recognizes the phosphorylated S114 in the RIIβ subunit of PKA. The orthologous phosphorylation site in mouse and rat PKA[RIIβ] is S112.



Analysis of PKA[RIIβ] (pS114) 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® 488 Mouse anti-PKA[RIIβ] (pS114). For data analysis, lymphocytes were selected by their scatter profile. The data demonstrates that the level of phosphorylation of PKA[RIIβ] 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® 488 under optimum conditions, and unreacted Alexa Fluor® 488 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), Bioimaging (Image), and western blot (WB) using these model systems:

Method	*Species*	*Cells*	*Treatment*	*Fixation*	*Perm buffer*	*Result*
Flow	Human	PBMC	Staurosporine	Cytofix	Perm I, II, or III	Down-regulation
Image	Human	SK-N-SH, SH-SY5Y	none	Cytofix	Perm III or Triton™ X-100	Cytoplasmic staining
	Rat	C6	none	Cytofix	Perm III or Triton™ X-100	Cytoplasmic staining
WB	Human	PBMC	Staurosporine			53-kDa band down-regulated
	Rat	Cerebrum	Lambda phosphatase			Loss of 53-kDa band

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. 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® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
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

Budillon A, Cereseto A, Kondrashin A, et al. Point mutation of the autophosphorylation site or in the nuclear location signal causes protein kinase A RII beta regulatory subunit to lose its ability to revert transformed fibroblasts. *Proc Natl Acad Sci U S A.* 1995; 92(23):10634-10638. (Biology)

Elliott MR, Shanks RA, Khan IU, et al. Down-regulation of IL-2 production in T lymphocytes by phosphorylated protein Kinase A-RIIβ. *J Immunol.* 2004; 172:7804-7812. (Biology)

Skalhegg BS, Tasken K. Specificity in the cAMP/PKA signaling pathway, differential expression, regulation, and subcellular localization of subunits of PKA. *Front Biosci.* 2000; 5:d678-d693. (Biology)