Revision 3

#5675 Store at -20C

PKA RI-α (D54D9) Rabbit mAb

Cell Signaling TECHNOLOGY®

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3 Trask Lane | Danvers | Massachusetts | 01923 | USA

For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP	H M R Hm Mk	Endogenous	48	Rabbit IgG	#P10644	5573

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

PKA RI-α (D54D9) Rabbit mAb recognizes endogenous levels of total PKA RI-α protein. This antibody may also detect PKA RI-β and detects a background band of unknown origin at 32 kDa.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gln371 of human PKA RI-α protein.

Background

The second messenger cyclic AMP (cAMP) activates cAMP-dependent protein kinase (PKA or cAPK) in mammalian cells and controls many cellular mechanisms such as gene transcription, ion transport, and protein phosphorylation (1). Inactive PKA is a heterotetramer composed of a regulatory subunit (R) dimer and a catalytic subunit (C) dimer. In this inactive state, the pseudosubstrate sequences on the R subunits block the active sites on the C subunits. Three C subunit isoforms (C-α, C-β, and C-γ) and two families of regulatory subunits (RI and RII) with distinct cAMP binding properties have been identified. The two R families exist in two isoforms, α and β (RI-α, RI-β, RII-α, and RII-β). Upon binding of cAMP to the R subunits, the autoinhibitory contact is eased and active monomeric C subunits are released. PKA shares substrate specificity with Akt (PKB) and PKC, which are characterized by an arginine at position -3 relative to the phosphorylated serine or threonine residue (2). Substrates that present this consensus sequence and have been shown to be phosphorylated by PKA are Bad (Ser155), CREB (Ser133), and GSK-3 (GSK-3α Ser21 and GSK-3β Ser9) (3-5). In addition, combined knock-down of PKA C-α and -β blocks cAMP-mediated phosphorylation of Raf (Ser43 and Ser259) (6). Autophosphorylation and phosphorylation by PDK-1 are two known mechanisms responsible for phosphorylation of the C subunit at Thr197 (7).

Background References

1. Montminy, M. (1997) *Annu. Rev. Biochem.* 66, 807-822.
2. Dell'Acqua, M.L. and Scott, J.D. (1997) *J. Biol. Chem.* 272, 12881-12884.
3. Tan, Y. et al. (2000) *J. Biol. Chem.* 275, 25865-25869.
4. Gonzalez, G.A. and Montminy, M.R. (1989) *Cell* 59, 675-680.
5. Fang, X. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 11960-11965.
6. Dumaz, N. and Marais, R. (2003) *J. Biol. Chem.* 278, 29819 -29823.
7. Moore, M.J. et al. (2002) *J. Biol. Chem.* 277, 47878-47884.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **IP:** Immunoprecipitation

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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