Technical Data Sheet

Purified Mouse Anti- PKA [RI]

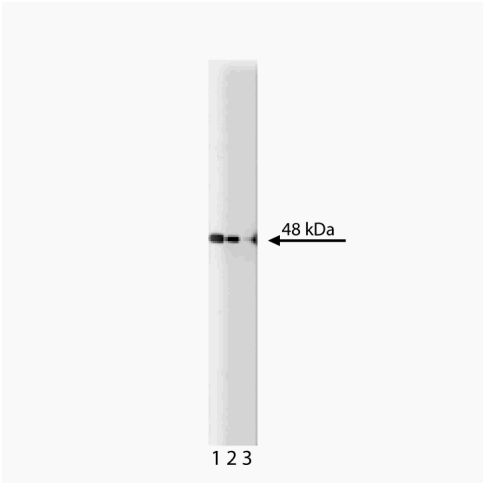
Product Information

Material Number:	**610165**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	18/PKA [RI]
Immunogen:	Mouse PKA [RI] subunit aa. 225-381
Isotype:	Mouse IgG2b
Reactivity:	QC Testing: Human Tested in Development: Chicken, Dog, Frog, Mouse, Rat
Target MW:	48 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

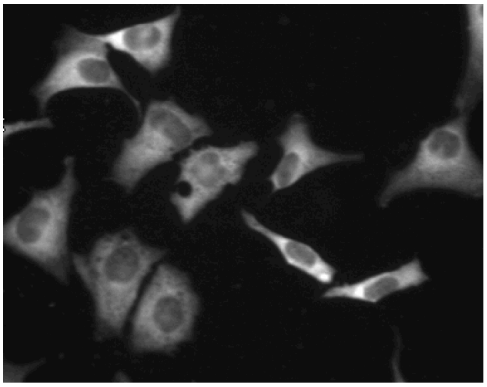
Description

cAMP-dependent **P**rotein **K**inase (PKA) is composed of two distinct subunits: catalytic (C) and regulatory (**R**). Four regulatory subunits have been identified: RIα, RIß, RIIα, and RIIß. These subunits define type I and II cAMP-dependent protein kinases. 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, Cß, or Cγ). Type II PKA can be distinguished by autophosphorylation of the R-subunits, while type I PKA binds Mg/ATP with high affinity. Most cells express both type I and type II PKAs. Although the Rα isoforms are ubiquitously expressed, the Rß isoforms are predominant in nervous and adipose tissues. The levels of expression of the different subunits vary according to cell and tissue type.

This antibody is routinely tested by Western blot analysis and immunofluorescent imaging. Other applications were tested at BD Bioscience Pharmingen during antibody development only.



Western blot analysis of PKA [RI] on a human endothelial lysate. *Lane 1: 1:250, lane 2: 1:500, lane 3: 1:1000 dilution of the PKA [RI] antibody.*



Immunofluorescent staining of A549 cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10 000 cells per well. After overnight incubation, cells were stained using the methanol fix/perm protocol (see Recommended Assay Procedure) and the anti-PKA [RI] antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a Pathway 850 imager using a 20x objective. This antibody also stained HeLa and U2OS cells and can be used with either fix/perm protocol (see Recommended Assay Procedure).*

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at -20° C.

Application Notes

Application

Bioimaging	Routinely Tested
Western blot	Routinely Tested
Immunofluorescence	Tested During Development
Immunoprecipitation	Tested During Development

Recommended Assay Procedure:

Methanol Procedure for a 96 well plate:
Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:
Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Suggested Companion Products

Catalog Number	Name	Size	Clone
611450	Human Endothelial Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Igs	1.0 ml	(none)
554001	FITC Goat Anti-Mouse Igs	0.5 mg	Polyclonal

Product Notices

1. 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.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Cho-Chung YS. Role of cyclic AMP receptor proteins in growth, differentiation, and suppression of malignancy: new approaches to therapy. *Cancer Res.* 1990; 50(22):7093-7100.(Biology)
Dohrman DP, Diamond I, Gordon AS. Ethanol causes translocation of cAMP-dependent protein kinase catalytic subunit to the nucleus. *Proc Natl Acad Sci U S A.* 1996; 93(19):10217-10221.(Clone-specific: Immunofluorescence)
Orellana SA, Marfella-Scivittaro C. Distinctive cyclic AMP-dependent protein kinase subunit localization is associated with cyst formation and loss of tubulogenic capacity in Madin-Darby canine kidney cell clones. *J Biol Chem.* 2000; 275(28):21233-21240.(Clone-specific: Immunofluorescence, Western blot)
Taylor SS, Buechler JA, Yonemoto W. cAMP-dependent protein kinase: framework for a diverse family of regulatory enzymes. *Annu Rev Biochem.* 1990; 59:971-1005.(Biology)