Technical Data Sheet

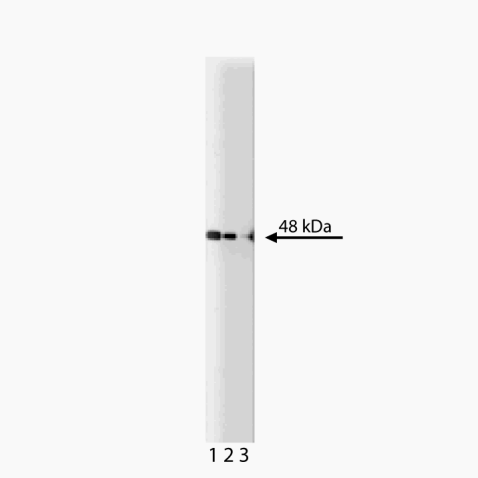
Purified Mouse Anti-PKA [RI]

Product Information

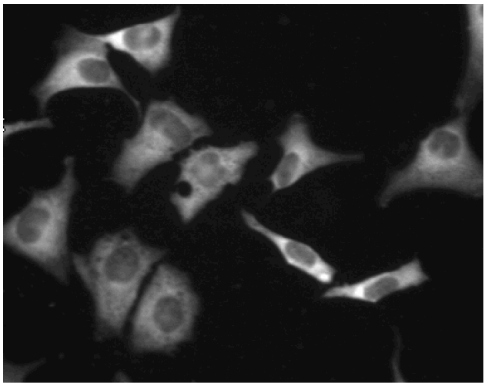
Material Number:	**610165**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	18/PKA [RI]
Immunogen:	Mouse PKA [RI] subunit aa. 225-381
Isotype:	Mouse IgG2b
Reactivity:	QC Testing: Human Tested in Development: Chicken, Dog, Frog, Mouse, Rat
Target MW:	48 kDa
Storage Buffer:	Aqueous buffered solution containing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

Description

cAMP-dependent Protein Kinase (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.



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 (ATCC CCL-185) 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 alcohol perm protocol 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 BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained HeLa (ATCC CCL-2) and U-2 OS (ATCC HTB-96) cells using both the Triton™ X-100 and alcohol perm protocol (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

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

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp
Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611450	Human Endothelial Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Triton is a trademark of the Dow Chemical Company.

References

Chen W, Yu YL, Lee SF, et al. CREB is one component of the binding complex of the Ces-2/E2A-HLF binding element and is an integral part of the interleukin-3 survival signal. *Mol Cell Biol.* 2001; 21(14):4636-4646. (Clone-specific: Flow cytometry)

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)