Bioimaging Certified Reagent

Technical Data Sheet

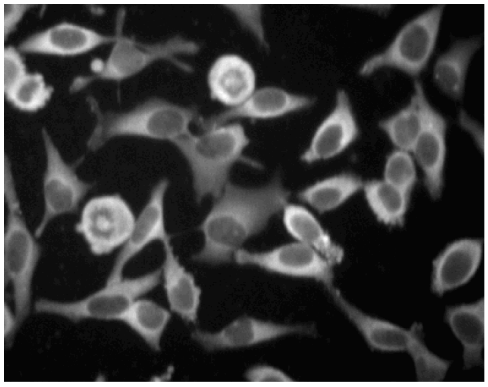
FITC Mouse Anti-PKA [RI]

Product Information

Material Number:	**610167**
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: Mouse, Rat, Dog, Chicken, Frog
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.



Immunofluorescent staining of HeLa cells. *HeLa cells (Human cervical epitheloid carcinoma; ATCC CCL-2.2) were seeded in a BD Falcon™ 96-well imaging plate (Cat. No. 353219) at ~ 10,000 cells per well. After overnight incubation, cells were stained using the Triton-X 100 fix/perm protocol (see Recommended Assay Procedure) and the FITC mouse anti-PKA [RI] antibody. The image was taken on a Pathway 855 imager using a 20x objective. This antibody also stained A549 and U2OS cells using either the Triton-X 100 or Methanol fix/perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.
Store undiluted at -20° C.

Application Notes

Application

Bioimaging	Routinely Tested
Immunofluorescence	Tested During Development

Recommended Assay Procedure:

Bioimaging: Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Bioimaging_Certified.shtml

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

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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
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)
610165	Purified Mouse Anti- PKA [RI]	50 µg	18/PKA [RI]

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. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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.(Biology)

Rohlff C, Clair T, Cho-Chung YS. 8-Cl-cAMP induces truncation and down-regulation of the RI alpha subunit and up-regulation of the RII beta subunit of cAMP-dependent protein kinase leading to type II holoenzyme-dependent growth inhibition and differentiation of HL-60 leukemia cells. *J Biol Chem.* 1993; 268(8):5774-5782.(Biology)

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)