Technical Data Sheet

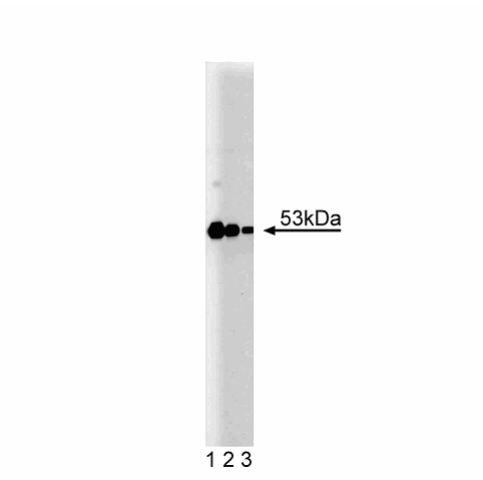
Purified Mouse Anti-PKA RIIβ

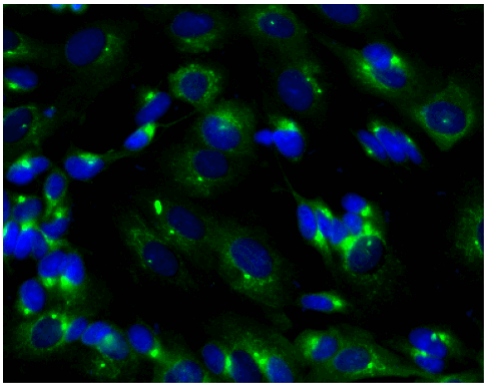
Product Information

Material Number:	**610625**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	45
Immunogen:	Human PKA RIIβ, aa 1-418
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Chicken, Dog, Mouse, Rat
Target MW:	53 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. There are indications that the deletion of the gene for PKA RIIß results in lack of long-term potentiation in a select group of hippocampal cells, suggesting an important role for this protein in the neurosciences.





Western blot analysis of PKA RIIβ on human endothelial lysate (left). *Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of PKA RIIβ.*

Immunofluorescent staining of SK-N-SH cells (right). ***Cells were seeded in a 384 well collagen coated Microplates (Material # 353962) at ~ 8,000 cells per well. After overnight incubation, cells were stained using the Triton X100 fix/perm protocol (see Recommended Assay Procedure) and the anti- PKARIIb antibody. The second step reagent was Alexa Fluor® 488 goat anti mouse Ig (Invitrogen). The image was taken on a Pathway 855 or 435 imager using a 20x objective. This antibody also stained SH-SY5Y and C6 cells using both the Triton X100 and 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.
Store undiluted at -20°C.

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Application Notes

Application

Western blot	Routinely Tested
Immunofluorescence	Tested During Development
Immunoprecipitation	Not Recommended
Immunohistochemistry	Not Recommended

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
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611450	Human Endothelial Cell Lysate	500 µg	(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. 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

Casey M, Vaughan CJ, He J, et al. Mutations in the protein kinase A R1alpha regulatory subunit cause familial cardiac myxomas and Carney complex. *J Clin Invest.* 2000; 106(5):R31-R38.(Clone-specific: Western blot)

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