abcam

Product datasheet

Anti-PKA R2/PKR2 antibody [Y116] ab32514

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-PKA R2/PKR2 antibody [Y116]
Description	Rabbit monoclonal [Y116] to PKA R2/PKR2
Host species	Rabbit
Specificity	The antibody recognises PKA RII. It does not cross-react with other cAMP-dependent kinase.
Tested applications	**Suitable for:** Flow Cyt (Intra), IP, WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human **Predicted to work with:** Cow
Immunogen	Synthetic peptide within Human PKA R2/PKR2 aa 350-450 (C terminal). The exact sequence is proprietary.
Positive control	WB: K562, HeLa and Jurkat cell lysates. IHC-P: Human colon cancer tissue. IP: K562 whole cell lysate. Flow Cyt (intra): MCF7 cells. ICC/IF: HeLa cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide

	Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y116
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32514 in the following tested applications.

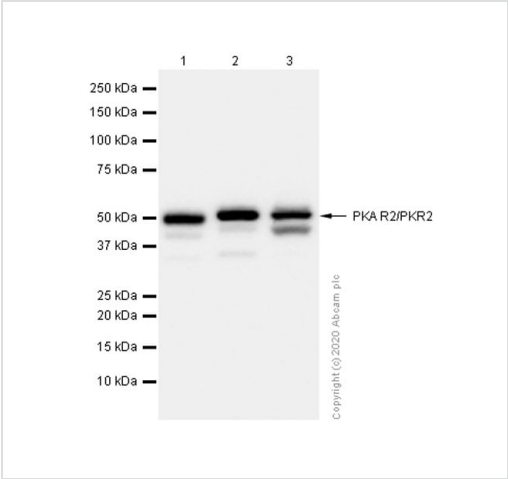
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/20.
IP		1/20.
WB	★☆☆☆☆ **(1)**	1/10000. Detects a band of approximately 52 kDa (predicted molecular weight: 45 kDa). **For unpurified use at 1/10000 - 1/50000.**
IHC-P		1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. **For unpurified use at 1/100 - 1/250.**
ICC/IF		1/500. **For unpurified use at 1/100 - 1/250.**

Target

Function	Regulatory subunit of the cAMP-dependent protein kinases involved in cAMP signaling in cells. Type II regulatory chains mediate membrane association by binding to anchoring proteins, including the MAP2 kinase.
Tissue specificity	Four types of regulatory chains are found: I-alpha, I-beta, II-alpha, and II-beta. Their expression varies among tissues and is in some cases constitutive and in others inducible.
Sequence similarities	Belongs to the cAMP-dependent kinase regulatory chain family. Contains 2 cyclic nucleotide-binding domains.
Post-translational modifications	Phosphorylated by the activated catalytic chain.
Cellular localization	Cytoplasm. Cell membrane. Colocalizes with PJA2 in the cytoplasm and the cell membrane.

Images



Western blot - Anti-PKA R2/PKR2 antibody [Y116] (ab32514)

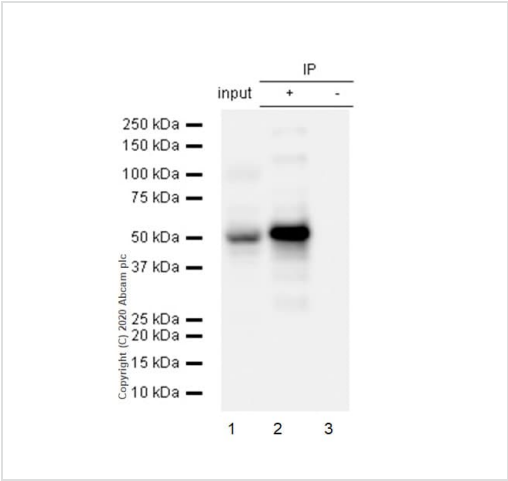
All lanes : Anti-PKA R2/PKR2 antibody [Y116] (ab32514) at 1/10000 dilution (Purified)

Lane 1 : K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 3 : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

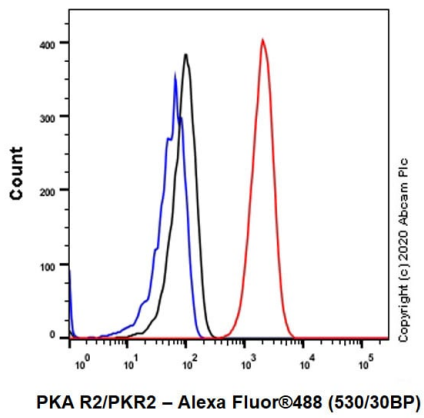
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 45 kDa
Observed band size: 50 kDa



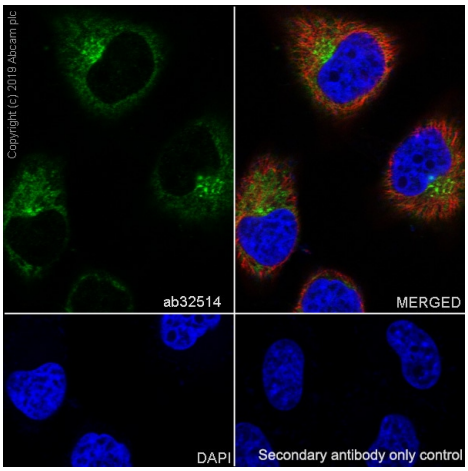
Immunoprecipitation - Anti-PKA R2/PKR2 antibody [Y116] (ab32514)

Purified ab32514 at 1/20 dilution (0.5µg) immunoprecipitating PKA R2/PKR2 in K-562 whole cell lysate.
Lane 1 (input): K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysate 10µg
Lane 2 (+): ab32514 + K-562 whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32514 in K-562 whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 50 kDa



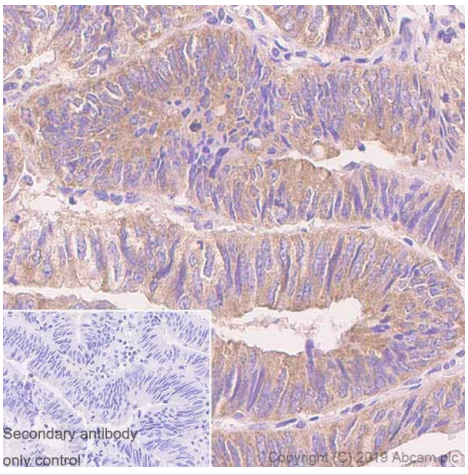
Flow Cytometry (Intracellular) - Anti-PKA R2/PKR2 antibody [Y116] (ab32514)

Intracellular Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling PKA R2/PKR2 with purified ab32514 at 1/20 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



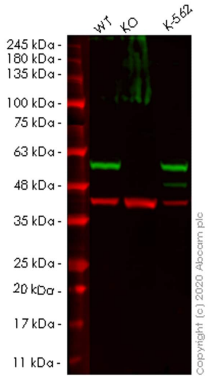
Immunocytochemistry/ Immunofluorescence - Anti-PKA R2/PKR2 antibody [Y116] (ab32514)

Immunocytochemistry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling PKA R2/PKR2 with purified ab32514 at 1/500 dilution (1.9 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PKA R2/PKR2 antibody [Y116] (ab32514)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon cancer tissue sections labeling PKA R2/PKR2 with purified ab32514 at 1/100 dilution (0.84 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-PKA R2/PKR2 antibody [Y116] (ab32514)

All lanes : Anti-PKA R2/PKR2 antibody [Y116] (ab32514) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PRKAR2A knockout HeLa cell lysate
Lane 3 : K-562 cell lysate

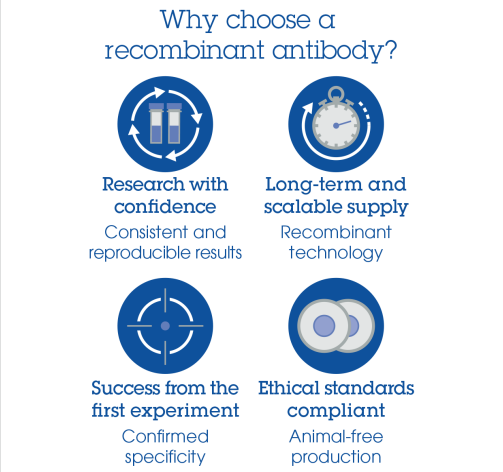
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 45 kDa
Observed band size: 50 kDa

Lanes 1-3: Merged signal (red and green). Green - ab32514 observed at 50 kDa. Red - loading control **ab8245** observed at 36 kDa.

Unpurified ab32514 Anti-PKA R2/PKR2 antibody [Y116] was shown to specifically react with PKA R2/PKR2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265748** (knockout cell lysate **ab257607**) was used. Wild-type and PKA R2/PKR2 knockout samples were subjected to SDS-PAGE. ab32514 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Anti-PKA R2/PKR2 antibody [Y116] (ab32514)

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