# abcam

# Product datasheet

# Anti-PP2A alpha + beta antibody [YE351] ab32065

Recombinant RobMAb

★★★★★ 1 Abreviews 5 References 13 Images

Overview

**Product name** Anti-PP2A alpha + beta antibody [YE351]

**Description** Rabbit monoclonal [YE351] to PP2A alpha + beta

Host species Rabbit

**Specificity** This antibody can bind both alpha and beta form of PP2A. Mouse samples have been

successfully tested in WB, but IHC-P sections from various mouse tissues showed negative

results.

**Tested applications** Suitable for: IP, WB, IHC-P, Dot blot

Unsuitable for: Flow Cyt or ICC/IF

**Species reactivity** Reacts with: Mouse, Rat, Human

Predicted to work with: Cow, Pig

Immunogen Synthetic peptide within Human PP2A alpha + beta (N terminal). The exact sequence is

proprietary.

**Epitope** ab32065 reacts with an epitope located in the N terminal region of PP2A alpha.

Positive control WB: A431 cell lysate and NIH 3T3 cell lysate. Dot Blot: PP2A-alpha peptide and PP2A-beta

peptide.

**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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

**Properties** 

Form Liquid

**Storage instructions** Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

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Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

**Purity** Protein A purified

Clonality Monoclonal

Clone number YE351

Isotype ΙgG

#### **Applications**

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab32065 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
IP		1/80.
WB	*** <u>*</u>	1/500. Predicted molecular weight: 36 kDa.
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Dot blot		Use at an assay dependent concentration.

**Application notes** Is unsuitable for Flow Cyt or ICC/IF.

#### **Target**

**Function** PP2A can modulate the activity of phosphorylase B kinase casein kinase 2, mitogen-stimulated

S6 kinase, and MAP-2 kinase. Cooperates with SGOL2 to protect centromeric cohesin from

separase-mediated cleavage in oocytes specifically during meiosis I (By similarity). Can

dephosphorylate SV40 large T antigen and p53/TP53. Dephosphorylates SV40 large T antigen, preferentially on serine residues 120, 123, 677, and perhaps 679. The C subunit was most active, followed by the AC form, which was more active than the ABC form, and activity of all three forms

was strongly stimulated by manganese, and to a lesser extent by magnesium. Dephosphorylation by the AC form, but not C or ABC form is inhibited by small T antigen. Activates RAF1 by

dephosphorylating it at 'Ser-259'.

Sequence similarities Belongs to the PPP phosphatase family. PP-1 subfamily.

Post-translational modifications

Reversibly methyl esterified on Leu-309. Carboxyl methylation may play a role in holoenzyme

assembly, enhancing the affinity of the PP2A core enzyme for some, but not all, regulatory

subunits. It varies during the cell cycle.

Phosphorylation of either threonine (by autophosphorylation-activated protein kinase) or tyrosine

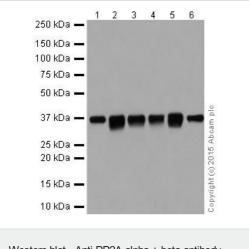
results in inactivation of the phosphatase. Auto-dephosphorylation has been suggested as a

mechanism for reactivation.

**Cellular localization** Cytoplasm. Nucleus. Chromosome > centromere. Cytoplasm > cytoskeleton > spindle pole. In

prometaphase cells, but not in anaphase cells, localizes at centromeres. During mitosis, also

found at spindle poles. Centromeric localization requires the presence of SGOL2.



Western blot - Anti-PP2A alpha + beta antibody [YE351] (ab32065)

**All lanes :** Anti-PP2A alpha + beta antibody [YE351] (ab32065) at 1/50000 dilution

Lane 1: A431 whole cell lysate
Lane 2: A549 whole cell lysate
Lane 3: Caco-2 whole cell lysate
Lane 4: T47D whole cell lysate

Lane 5 : C2C12 whole cell lysate

Lane 6 : HeLa whole cell lysate

Lysates/proteins at 10 µg per lane.

#### **Secondary**

**All lanes :** Peroxidase-conjugated goat anti-rabbit lgG (H+L) at 1/1000 dilution

**Predicted band size:** 36 kDa **Observed band size:** 36 kDa

Exposure time: 15 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

250 kDa — 1 2
150 kDa — 100 kDa — 75 kDa — 50 kDa — 37 kDa — 25 kDa — 20 kDa — 15 kDa — 10 kD

Western blot - Anti-PP2A alpha + beta antibody [YE351] (ab32065)

**All lanes :** Anti-PP2A alpha + beta antibody [YE351] (ab32065) at 1/50000 dilution

Lane 1 : Jurkat whole cell lysate
Lane 2 : U-937 whole cell lysate

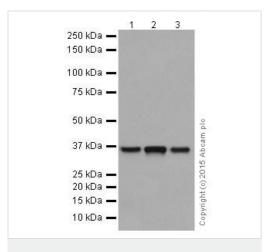
Lysates/proteins at 10 µg per lane.

#### **Secondary**

**All lanes :** Peroxidase-conjugated goat anti-rabbit lgG (H+L) at 1/1000 dilution

**Predicted band size:** 36 kDa **Observed band size:** 36 kDa

Exposure time: 5 seconds



Western blot - Anti-PP2A alpha + beta antibody [YE351] (ab32065)

Blocking and dilution buffer: 5% NFDM/TBST.

**All lanes :** Anti-PP2A alpha + beta antibody [YE351] (ab32065) at 1/100000 dilution

Lane 1 : Human fetal heart tissue lysate
Lane 2 : Human fetal kidney tissue lysate
Lane 3 : Human fetal spleen tissue lysate

Lysates/proteins at 10 µg per lane.

#### **Secondary**

**All lanes :** HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of lgG at 1/1000 dilution

**Predicted band size:** 36 kDa **Observed band size:** 36 kDa

Exposure time: 30 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

1 250 kDa — 150 kDa — 150 kDa — 25 kDa — 20 kDa — 15 kDa — 15 kDa — 15 kDa — 10 kDa

Western blot - Anti-PP2A alpha + beta antibody [YE351] (ab32065)

Anti-PP2A alpha + beta antibody [YE351] (ab32065) at 1/100000 dilution + Human fetal brain tissue lysate at 10 µg

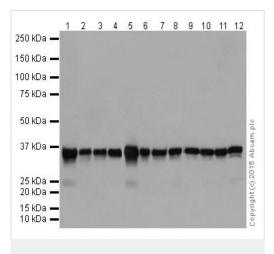
### Secondary

HRP-conjugate anti-rabbit IgG, specific to the non-reduced form of IgG at 1/1000 dilution

**Predicted band size:** 36 kDa **Observed band size:** 36 kDa

Exposure time: 15 seconds

Blocking and dilution buffer: 5% NFDM /TBST.



Western blot - Anti-PP2A alpha + beta antibody [YE351] (ab32065)

**All lanes :** Anti-PP2A alpha + beta antibody [YE351] (ab32065) at 1/100000 dilution

Lane 1: Mouse brain tissue lysate

Lane 2: Mouse heart tissue lysate

Lane 3: Mouse kidney tissue lysate

Lane 4: Mouse spleen tissue lysate

Lane 5: Rat brain tissue lysate

Lane 6: Rat heart tissue lysate

Lane 7: Rat kidney tissue lysate

Lane 8: Rat spleen tissue lysate

Lane 9: C6 whole cell lysate

Lane 10: Raw264.7 whole cell lysate

Lane 11: PC-12 whole cell lysate

Lane 12: NIH/3T3 whole cell lysate

Lysates/proteins at 10 µg per lane.

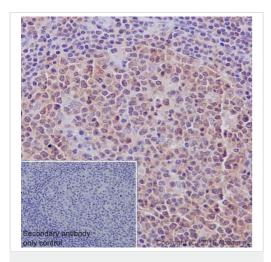
#### **Secondary**

**All lanes :** Peroxidase-conjugated goat anti-rabbit lgG (H+L) at 1/1000 dilution

**Predicted band size:** 36 kDa **Observed band size:** 36 kDa

Exposure time: 1 second

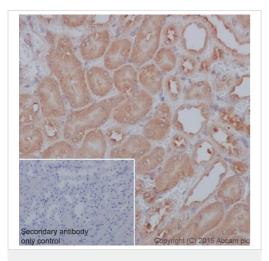
Blocking and dilution buffer: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PP2A alpha + beta antibody [YE351] (ab32065)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labeling PP2A alpha + beta with ab32065 at a dilution of 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <a href="mailto:ab97051">ab97051</a>, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

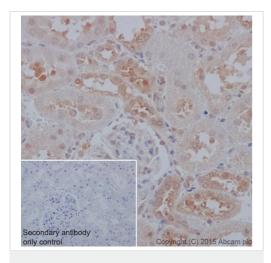
Cytoplasm and weak nucleus staining is visible in lymphocytes of human tonsil.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PP2A alpha + beta antibody [YE351] (ab32065)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue labelling PP2A alpha + beta with ab32065 at a dilution of 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <a href="mailto:ab97051">ab97051</a>, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

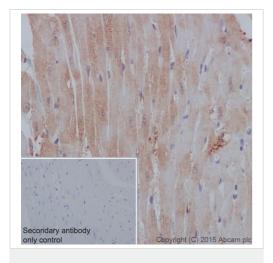
Cytoplasm staining is visible in epithelial cells of mouse kidney



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PP2A alpha + beta antibody [YE351] (ab32065)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat kidney tissue labelling PP2A alpha + beta with ab32065 at a dilution of 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <a href="mailto:ab97051">ab97051</a>, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

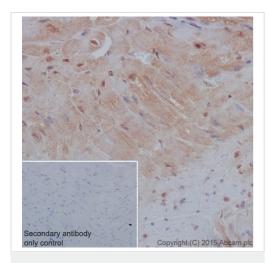
Cytoplasm and weak nucleus staining is visible in epithelial cells of rat kidney.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PP2A alpha + beta antibody [YE351] (ab32065)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse heart tissue labelling PP2A alpha + beta with ab32065 at a dilution of 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <a href="mailto:ab97051">ab97051</a>, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

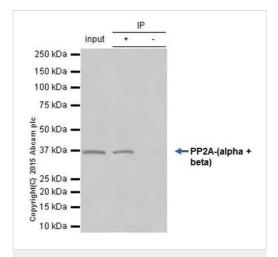
Cytoplasm staining is visible in mouse heart.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PP2A alpha + beta antibody [YE351] (ab32065)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat heart tissue labelling PP2A alpha + beta with ab32065 at a dilution of 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. <a href="mailto:ab97051">ab97051</a>, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Cytoplasm and nucleus staining is visible on rat heart.



Immunoprecipitation - Anti-PP2A alpha + beta antibody [YE351] (ab32065)

ab32065 at 1/80 immunoprecipitating PP2A alpha + beta in HeLa whole cell lysate.

Lane 1 (input): HeLa whole cell lysate (10µg)

Lane 2 (+): ab32065 + HeLa whole cell lysate.

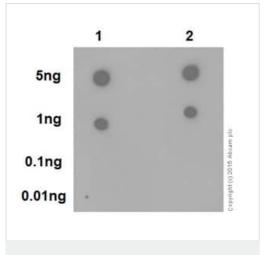
Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab32065 in HeLa (human cervix adenocarcinoma) whole cell lysate.

For western blotting, ab32065 (1/500) <u>ab131366</u> VeriBlot for IP Detection Reagent (HRP) was used for detection (1/10000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

Observed band: 37kDa.

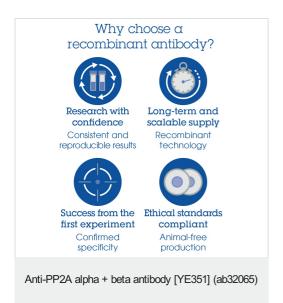


Dot Blot - Anti-PP2A alpha + beta antibody [YE351] (ab32065)

Dot blot analysis of PP2A-alpha peptide (Lane 1) and PP2A-beta peptide (Lane 2) labelling PP2A alpha+beta with ab32065 at a dilution of 1/1000. A HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of lgG was used as the secondary antibody at a dilution of 1/1000.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



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