

Anti-RPA32/RPA2 (phospho T21) antibody [EPR2846(2)] ab109394

Recombinant RabMAb

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Overview

Product name	Anti-RPA32/RPA2 (phospho T21) antibody [EPR2846(2)]
Description	Rabbit monoclonal [EPR2846(2)] to RPA32/RPA2 (phospho T21)
Host species	Rabbit
Specificity	<p>ab109394 only detects RPA32/RPA2 phosphorylated at threonine 21. The antibody has been tested with milk (5%) and BSA (2%) as blocking reagents, but it provided better results with 5% milk.</p> <p>This antibody cross-reactivates with non-phospho substrate in specific situations.</p>
Tested applications	<p>Suitable for: Dot blot, WB, ELISA</p> <p>Unsuitable for: Flow Cyt, ICC/IF or IHC-P</p>
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa whole cell lysate treated with Calyculin A or Camptothecin. Dot Blot: RPA32/RPA2 (pT21) phospho peptide.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20

	Preservative: 0.05% Sodium azide
	Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue culture supernatant
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR2846(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109394 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

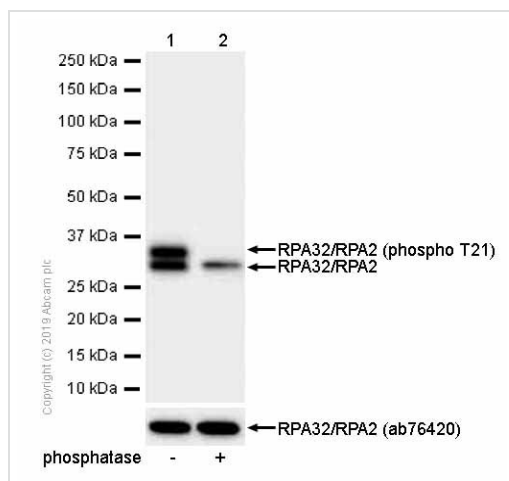
Application	Abreviews	Notes
Dot blot		1/1000.
WB		1/50000 - 1/200000. Detects a band of approximately 32 kDa (predicted molecular weight: 29 kDa). The antibody has been tested with milk (5%) and BSA (2%) as blocking reagents, but it provided better results with 5% milk.
ELISA		Use at an assay dependent concentration.

Application notes Is unsuitable for Flow Cyt, ICC/IF or IHC-P.

Target

Function	Required for DNA recombination, repair and replication. The activity of RP-A is mediated by single-stranded DNA binding and protein interactions. Functions as component of the alternative replication protein A complex (aRPA). aRPA binds single-stranded DNA and probably plays a role in DNA repair; it does not support chromosomal DNA replication and cell cycle progression through S-phase. In vitro, aRPA cannot promote efficient priming by DNA polymerase alpha but supports DNA polymerase delta synthesis in the presence of PCNA and replication factor C (RFC), the dual incision/excision reaction of nucleotide excision repair and RAD51-dependent strand exchange.
Post-translational modifications	Phosphorylated in a cell-cycle-dependent manner (from the S phase until mitosis). Phosphorylated by ATR upon DNA damage, which promotes its translocation to nuclear foci. Can be phosphorylated in vitro by PRKDC/DNA-PK in the presence of Ku and DNA, and by CDK1.
Cellular localization	Nucleus. Nucleus > PML body. Also present in PML nuclear bodies. Redistributes to discrete nuclear foci upon DNA damage.

Images



Western blot - Anti-RPA32/RPA2 (phospho T21) antibody [EPR2846(2)] (ab109394)

All lanes : Anti-RPA32/RPA2 (phospho T21) antibody [EPR2846(2)] (ab109394) at 1/5000 dilution

Lane 1 : MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysates

Lane 2 : MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysates. Then the membrane was incubated with phosphatase.

Lysates/proteins at 15 µg per lane.

Secondary

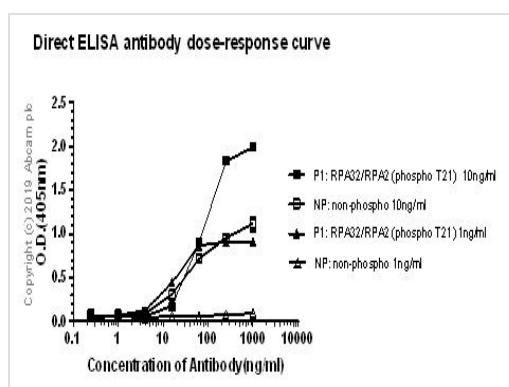
All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Predicted band size: 29 kDa

Observed band size: 32,36 kDa

Exposure time: 30 seconds

Blocking/Diluting buffer and concentration: 5% NFDM/TBST



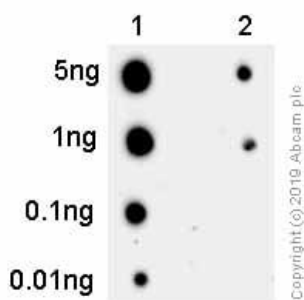
ELISA - Anti-RPA32/RPA2 (phospho T21) antibody [EPR2846(2)] (ab109394)

Direct ELISA antigen dose-response curve using purified ab109394.

Primary antibody: ab109394 (0-1000nweg/ml).

Antigens: P1: Human RPA32/RPA2 (phospho T21) at 10ng/ml and 1ng/ml; NP: non-phospho at 10ng/ml and 1 ng/ml.

Secondary antibody: Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) at 1/2500 dilution.

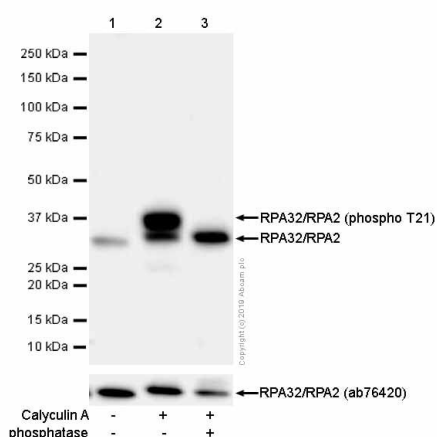


Dot Blot - Anti-RPA32/RPA2 (phospho T21) antibody
[EPR2846(2)] (ab109394)

Dot blot analysis of human c-Myb RPA32/RPA2 (pT21) phosphopeptide (Lane 1) and RPA32/RPA2 non-phospho peptide (Lane 2) labeling RPA32/RPA2 (phospho T21) with purified ab109394 at a dilution of 1/5000. **ab97051** (Peroxidase conjugated goat anti-rabbit IgG (H+L)) was used as the secondary antibody at a dilution of 1/2500.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Western blot - Anti-RPA32/RPA2 (phospho T21)
antibody [EPR2846(2)] (ab109394)

All lanes : Anti-RPA32/RPA2 (phospho T21) antibody
[EPR2846(2)] (ab109394) at 1/2000 dilution

Lane 1 : C6 (Rat glial tumor glial cell) whole cell lysates

Lane 2 : C6 (Rat glial tumor glial cell) treated with 100nM calyculin A for 60 minutes whole cell lysates

Lane 3 : C6 (Rat glial tumor glial cell) treated with 100nM calyculin A for 60 minutes whole cell lysates. Then the membrane was incubated with phosphatase.

Lysates/proteins at 15 µg per lane.

Secondary

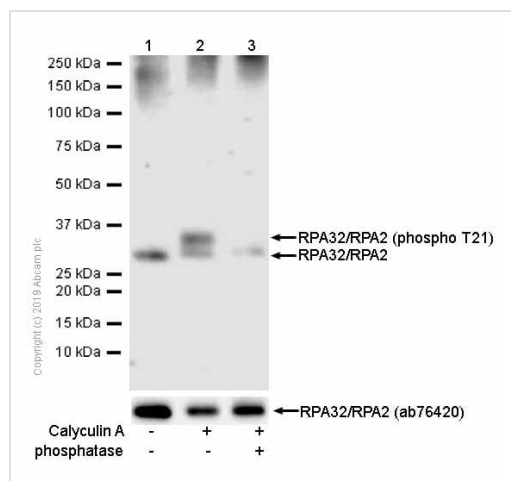
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 29 kDa

Observed band size: 32,36 kDa

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 110 seconds.



Western blot - Anti-RPA32/RPA2 (phospho T21) antibody [EPR2846(2)] (ab109394)

All lanes : Anti-RPA32/RPA2 (phospho T21) antibody [EPR2846(2)] (ab109394) at 1/2000 dilution

Lane 1 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) treated with 100ng/ml calyculin A for 30 minutes whole cell lysates

Lane 3 : NIH/3T3 (Mouse embryonic fibroblast) treated with 100ng/ml calyculin A for 60 3minutes whole cell lysates. Then the membrane was incubated with phosphatase

Lysates/proteins at 15 µg per lane.

Secondary

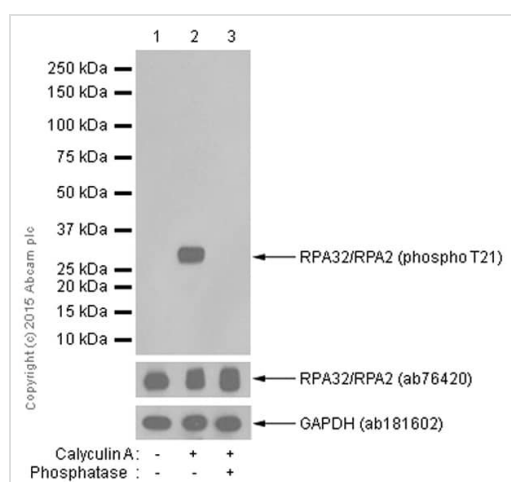
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 29 kDa

Observed band size: 32,36 kDa

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 180 seconds.



Western blot - Anti-RPA32/RPA2 (phospho T21) antibody [EPR2846(2)] (ab109394)

All lanes : Anti-RPA32/RPA2 (phospho T21) antibody [EPR2846(2)] (ab109394) at 1/50000 dilution

Lane 1 : HeLa whole cell lysate - untreated

Lane 2 : HeLa whole cell lysate - treated with Calyculin A

Lane 3 : HeLa whole cell lysate - treated with Calyculin A and Alkaline Phosphatase

Lysates/proteins at 10 µg per lane.

Secondary

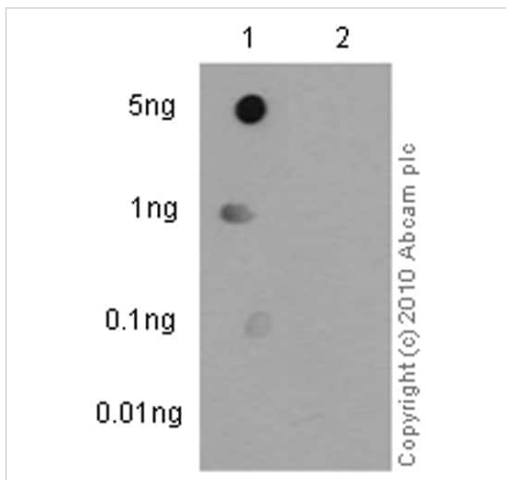
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 29 kDa

Observed band size: 32 kDa

Exposure time: 30 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

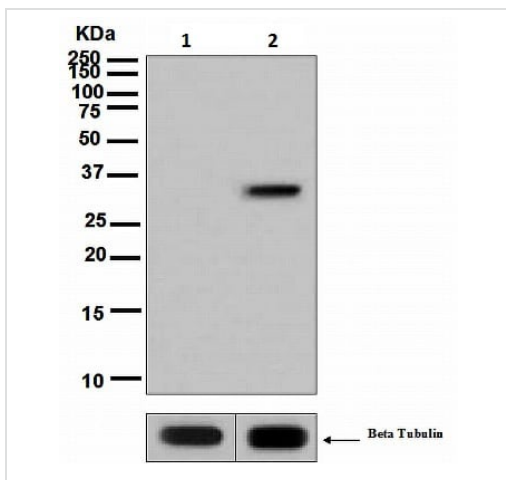


Dot Blot - Anti-RPA32/RPA2 (phospho T21) antibody
[EPR2846(2)] (ab109394)

Dot blot analysis of RPA32/RPA2 (pT21) phospho peptide (lane 1) and RPA32/RPA2 non-phospho peptide (lane 2) labelling RPA32/RPA2 (phospho T21) with ab109394 at a dilution of 1/1000. A peroxidase-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/2500).

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Western blot - Anti-RPA32/RPA2 (phospho T21) antibody [EPR2846(2)] (ab109394)

All lanes : Anti-RPA32/RPA2 (phospho T21) antibody [EPR2846(2)] (ab109394) at 1/50000 dilution

Lane 1 : HeLa cell lysates, untreated

Lane 2 : HeLa cell lysates, treated with Camptothecin

Lysates/proteins at 10 µg per lane.

Predicted band size: 29 kDa

Observed band size: 32 kDa

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-RPA32/RPA2 (phospho T21) antibody
[EPR2846(2)] (ab109394)

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