## abcam

### Product datasheet

# Anti-RIP3 (phospho T231 + S232) antibody [EPR19403-52] ab222320



Recombinant

RabMAb

16 References 4 Images

### Overview

Product name Anti-RIP3 (phospho T231 + S232) antibody [EPR19403-52]

**Description** Rabbit monoclonal [EPR19403-52] to RIP3 (phospho T231 + S232)

Host species Rabbit

Tested applications Suitable for: WB, Dot blot

Species reactivity Reacts with: Mouse

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: L-929 treated with 20 ng/ml TNF-a, 100 nM Smac mimetic and 20 μM z-VAD for 6 hr, whole

cell lysate.

**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. 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: 7.2

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol, PBS

Purity Protein A purified

Clonality Monoclonal

Clone number EPR19403-52

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**Isotype** IgG

### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab222320 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
WB		1/1000. Detects a band of approximately 53 kDa (predicted molecular weight: 57 kDa).
Dot blot		1/1000.

### **Target**

**Function** Promotes apoptosis.

**Tissue specificity** Detected at lower levels in heart, placenta, lung and kidney. Isoform 3 is significantly increased in

colon and lung cancers.

**Sequence similarities**Belongs to the protein kinase superfamily. TKL Ser/Thr protein kinase family.

Contains 1 protein kinase domain.

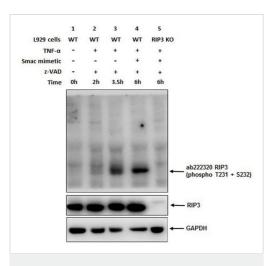
Post-translational

modifications

Autophosphorylated.

Cellular localization Cytoplasm.

### **Images**



Western blot - Anti-RIP3 (phospho T231 + S232) antibody [EPR19403-52] (ab222320)

**All lanes :** Anti-RIP3 (phospho T231 + S232) antibody [EPR19403-52] (ab222320) at 1/500 dilution

**Lane 1 :** Untreated L-929 (mouse connective tissue fibroblast cell line) whole cell lysate

**Lane 2 :** L-929 (mouse connective tissue fibroblast cell line) whole cell lysate treated with 10 ng/ml TNF-a and 20  $\mu$ M z-VAD for 2 hours

**Lane 3 :** L-929 (mouse connective tissue fibroblast cell line) whole cell lysate treated with 10 ng/ml TNF-a and 20  $\mu$ M z-VAD for 3.5 hours

**Lane 4 :** L-929 (mouse connective tissue fibroblast cell line) whole cell lysate treated with 20 ng/ml TNF-a, 100 nM Smac mimetic and 20  $\mu$ M z-VAD for 6 hours

**Lane 5 :** L-929 (mouse connective tissue fibroblast cell line) whole cell lysate with RIP3 KO cells treated with 20 ng/ml TNF-a, 100 nM Smac mimetic and 20  $\mu$ M z-VAD for 6 hours

Lysates/proteins at 20 µg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/5000 dilution

Predicted band size: 57 kDa

Exposure time: 60 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

Cells were lysed in 4% SDS buffer (4% SDS,10% 2-ME, 20% glycerol, 0.004% Bromophenol blue, Tris-HCI).

The images were kindly provided by our collaborator Dr. Jiahuai Han, Xiamen University.

**All lanes**: Anti-RIP3 (phospho T231 + S232) antibody [EPR19403-52] (ab222320) at 1/1000 dilution

**Lane 1 :** Untreated L-929 (mouse connective tissue fibroblast cell line) whole cell lysate

**Lane 2 :** L-929 (mouse connective tissue fibroblast cell line) whole cell lysate treated with 20 ng/ml TNF-a, 100 nM Smac mimetic and 20  $\mu$ M z-VAD for 6 hours

**Lane 3 :** L-929 (mouse connective tissue fibroblast cell line) whole cell lysate treated with 20 ng/ml TNF-a, 100 nM Smac mimetic and 20  $\mu$ M z-VAD for 6 hours, then treated with alkaline phosphatase for 1 hour

Lysates/proteins at 10 µg per lane.

# 1 2 3 250 kDa — 150 kDa — 100 kDa — 75 kDa — 37 kDa — 37 kDa — 25 kDa — 225 kDa — 24 kDa — 25 kDa — 26 kDa — 26 kDa — 27 kDa — 28 kDa — 29 kDa — 29 kDa — 29 kDa — 20 kDa — 20 kDa — 20 kDa — 21 kDa — 22 kDa — 24 kDa — 25 kDa — 26 kDa — 27 kDa — 28 kDa — 29 kDa — 29 kDa — 20 kDa — 20 kDa — 20 kDa — 21 kDa — 22 kDa — 24 kDa — 25 kDa — 26 kDa — 27 kDa — 28 kDa — 29 kDa — 29 kDa — 29 kDa — 20 kDa — 20 kDa — 21 kDa — 22 kDa — 24 kDa — 25 kDa — 26 kDa — 27 kDa — 28 kDa — 28 kDa — 29 kDa — 29 kDa — 20 kDa — 21 kDa — 22 kDa — 24 kDa — 25 kDa — 26 kDa — 27 kDa — 28 kDa — 2

Western blot - Anti-RIP3 (phospho T231 + S232) antibody [EPR19403-52] (ab222320)

### **Secondary**

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

Predicted band size: 57 kDa
Observed band size: 53 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The higher MW bands in Lanes 1 and 2 are extra bands.

The lysates were prepared following the 1% SDS Hot Lysate buffer method.

For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy)**.

Dot blot analysis of RIP3 (phospho T231 + S232) labeled with ab222320 at 1/1000 dilution.

Lane 1: RIP3 (phospho T231 + S232) peptide (aa229-239);

Lane 2: RIP3 (phospho T231 + S232) peptide (aa223-236);

Lane 3: RIP3 (phospho T231) peptide (aa223-239);

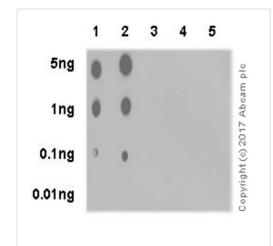
Lane 4: RIP3 (phospho S232) peptide (aa223-239);

Lane 5: RIP3 non-phospho peptide (aa223-239).

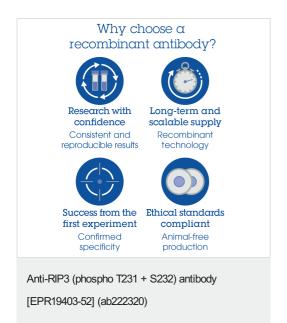
Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution was used as secondary antibody.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Dot Blot - Anti-RIP3 (phospho T231 + S232) antibody [EPR19403-52] (ab222320)



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