abcam

Product datasheet

Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] ab214036

Recombinant RabMAb

22 References 15 Images

Overview

Product name Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444]

Description Rabbit monoclonal [EPR18444] to ERK1 (phospho T202) + ERK2 (phospho T185)

Host species Rabbit

Specificity ab214036 does not react with a peptide containing ERK1 pY204 or ERK2 pY187

Tested applications Suitable for: Dot blot, IHC-P, WB, ICC/IF, IP

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: Jurkat treated with 200 ng/ml PMA for 30 minutes whole cell lysate; NIH/3T3 treated with 50

ng/ml PDGF for 40 minutes whole cell lysate; PC-12 treated with 200 ng/ml NGF for 4 days whole cell lysate. IHC-P: Human breast, placenta, breast cancer and glioma tissues; mouse kidney tissue; rat spleen tissue. ICC/IF: Jurkat cells treated with PMA treatment (200 ng/ml, 30min). IP: Jurkat treated with 200 ng/ml PMA for 30 minutes cell lysate; PC-12 treated with 200 ng/ml NGF

for 4 days cell lysate.

General notesThis 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**.

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

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Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR18444

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab214036 in the following tested applications.

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

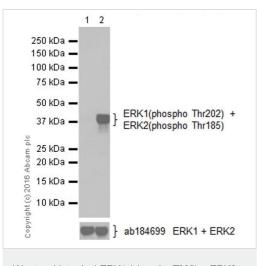
Application	Abreviews	Notes	
Dot blot		Use at an assay dependent concentration.	
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.	
WB		1/1000. Detects a band of approximately 44, 42 kDa (predicted molecular weight: 43, 41 kDa).	
ICC/IF		1/100.	
IP		1/40.	

Target

Cellular localization

ERK2: Nucleus.

Images



Western blot - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

All lanes : Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036) at 1/1000 dilution

Lane 1 : Untreated Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lane 2: Jurkat (Human T cell leukemia cell line from peripheral blood) treated with 200 ng/ml PMA for 30 minutes whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 43, 41 kDa Observed band size: 42,44 kDa

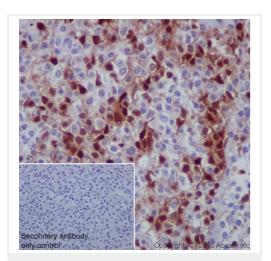
Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

	Ab214036 Anti-ERK1(phospho Thr202) + ERK2(phospho Thr185)		Ab184699 Anti-ERK1 + ERK2	
Untreated Jurkat	Ab214036	Ab214036+DAPI-tubulin	Ab184699	Ab184699+DAPI+tubulin
Jurkat+PMA	,8 6% 0 %		3 83	
Jurkat+PMA +LP	6	100°		pyright (c) 2016 Abcam plo

Immunocytochemistry/ Immunofluorescence - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036) Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cell line from peripheral blood) cells labeling ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining increased after PMA treatment (200 ng/ml, 30min), and LP treatment decreased the PMA induced staining. For the "pan" antibody, the signal is unchanged after PMA treatment (200 ng/ml, 30min), and LP treatment. The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) (ab150120) secondary antibody at 1/1000 dilution (red).



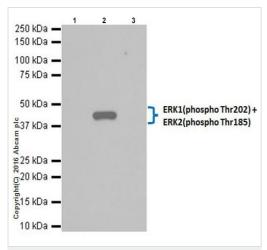
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

Immunohistochemical analysis of paraffin-embedded human glioma tissue labeling ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

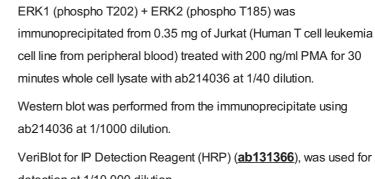
Nuclear and weak cytoplasmic staining on human glioma is observed [PMID:17487353].

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.



Immunoprecipitation - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)



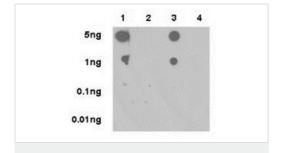
VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10.000 dilution

Lane 1: Jurkat treated with 200 ng/ml PMA for 30 minutes whole cell lysate 10µg (Input).

Lane 2: ab214036 IP in Jurkat treated with 200 ng/ml PMA for 30 minutes whole cell lysate.

Lane 3: Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) instead of ab214036 in Jurkat treated with 200 ng/ml PMA for 30 minutes whole cell lysate.

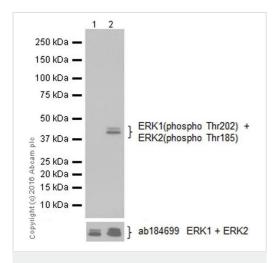
Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 3 minutes.



Dot Blot - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

Dot blot analysis of ERK1 (pT202) peptide (Lane 1), ERK1 (pT204) peptide (Lane 2), ERK1 (pT202 + pT204) peptide (Lane 3) and ERK1 non-phospho peptide (Lane 4) labelling ERK1 (pT202) with ab214036.

Exposure time: 3 minutes.



Western blot - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

All lanes: Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036) at 1/1000 dilution

Lane 1 : Untreated NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lane 2: NIH/3T3 (Mouse embryonic fibroblast cell line) treated with 50 ng/ml PDGF for 40 minutes whole cell lysate

Lysates/proteins at 10 µg per lane.

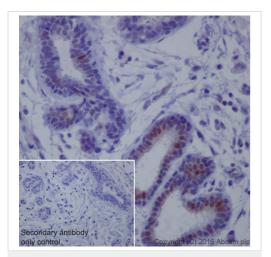
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 43, 41 kDa **Observed band size:** 42,44 kDa

Exposure time: 2 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



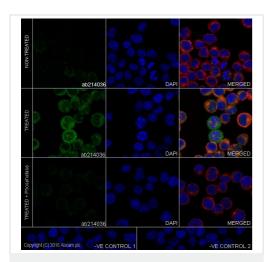
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

Immunohistochemical analysis of paraffin-embedded human breast tissue labeling ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

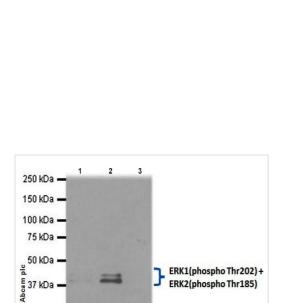
Nuclear staining on human normal breast tissue is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)



Immunoprecipitation - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

25 kDa

© 20 kDa

置15 kDa

ි 10 kDa

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cell line from peripheral blood) cells labeling -ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/100 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining increased after PMA treatment (200 ng/ml, 30min), and LP treatment decreased the PMA induced staining.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb ($\underline{ab7291}$) at 1/1000 dilution, followed by Anti-Mouse IgG H&L (Alexa Fluor® 594) ($\underline{ab150120}$) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab214036 at 1/100 dilution followed by Anti-Mouse lgG H&L (Alexa Fluor[®] 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (<u>ab7291</u>) at 1/1000 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution.

ERK1 (phospho T202) + ERK2 (phospho T185) was immunoprecipitated from 0.35 mg of PC-12 (Rat adrenal gland pheochromocytoma cell line) treated with 200 ng/ml NGF for 4 days whole cell lysate with ab214036 at 1/40 dilution.

Western blot was performed from the immunoprecipitate using ab214036 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10,000 dilution

Lane 1: PC-12 treated with 200 ng/ml NGF for 4 days whole cell lysate $10\mu g$ (lnput).

Lane 2: ab214036 IP in PC-12 treated with 200 ng/ml NGF for 4 days whole cell lysate.

Lane 3: Rabbit lgG, monoclonal [EPR25A] - Isotype Control (ab172730) instead of ab214036 in PC-12 treated with 200 ng/ml NGF for 4 days whole cell lysate.

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

1 2
250 kDa —
150 kDa —
100 kDa —
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50 kDa —
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37 kDa —
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16 kDa —
17 kDa —
18 kDa —
19 kDa —
19 kDa —
10 kD

Western blot - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

Exposure time: 30 seconds.

All lanes : Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036) at 1/1000 dilution

Lane 1 : Untreated PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lane 2: PC-12 (Rat adrenal gland pheochromocytoma cell line) treated with 200 ng/ml NGF for 4 days whole cell lysate

Lysates/proteins at 10 µg per lane.

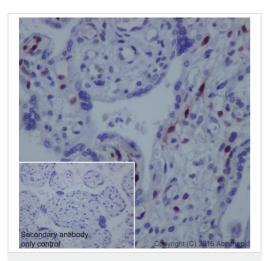
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 43, 41 kDa Observed band size: 42,44 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



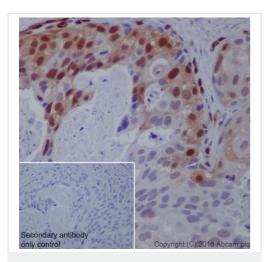
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

Immunohistochemical analysis of paraffin-embedded human placenta tissue labeling ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nuclear and weak cytoplasmic staining on scattered cells of human placenta is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

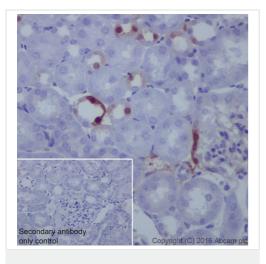
Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nuclear and weak cytoplasmic staining on human breast tissue cancer is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



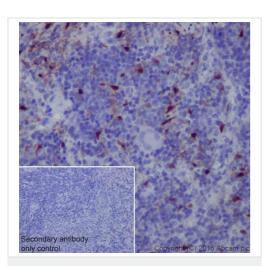
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nuclear and weak cytoplasmic staining on scattered cells of mouse kidney is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

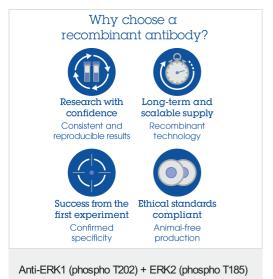
Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Nuclear and weak cytoplasmic staining on scattered cells of rat spleen is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



antibody [EPR18444] (ab214036)

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