abcam

Product datasheet

Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR19401] ab201015



★★★★★ 4 Abreviews 157 References 9 Images

Overview

Product name Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR19401]

Rabbit monoclonal [EPR19401] to ERK1 (phospho T202) + ERK2 (phospho T185) **Description**

Host species Rabbit

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

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, untreated and

> treated with 50 ng/ml PDGF for 40 minutes whole cell lysates; PC-12, untreated and treated with 200 ng/ml NGF for 4 days whole cell lysates. IHC-P: Human breast and glioma tissues. IP: NIH/3T3 treated with 50ng/ml PDGF for 40min whole cell lysate; PC-12 treated with 100ng/ml

NGF for 10min whole cell lysate. ICC/IF:Jurkat

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**.

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: 59% PBS, 0.05% BSA, 40% Glycerol

Purity Protein A purified

Clonality Monoclonal

Clone number EPR19401

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab201015 in the following tested applications.

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

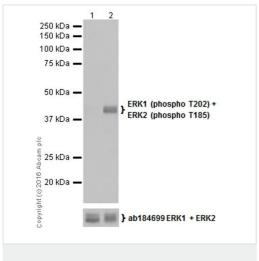
Application	Abreviews	Notes
ICC/IF	★★★★ (3)	Use at an assay dependent concentration.
IP		1/30.
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. IHC is recommended for human only.
WB	**** (1)	1/1000. Detects a band of approximately 44, 42 kDa (predicted molecular weight: 41 kDa).
Dot blot		1/1000.

Target

Cellular localization

ERK2: Nucleus.

Images



Western blot - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR19401] (ab201015)

All lanes: Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR19401] (ab201015) 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 20 µg per lane.

Secondary

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

Predicted band size: 41 kDa

Observed band size: 42,44 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

AD201015 DAPI MERGED

AD201015 DAPI MERGED

AD201015 DAPI MERGED

CONTROL 1

CONTROL 2

***CONTROL 2**

*

Immunocytochemistry/ Immunofluorescence - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR19401] (ab201015)

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) and ERK2 (phospho T185)

ERK1 (phospho T202) + ERK2 (phospho T185) with ab201015 at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green).

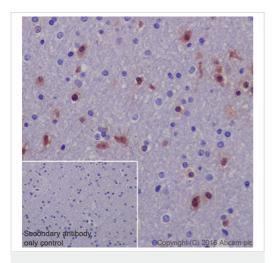
Confocal image showing staining on M phase cells (PMID:26529125). After PMA treatment (200 ng/ml, 30min), the staining was increased, and LP treatment decreased the PMA induced staining.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (Alexa Fluor[®] 594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab201015 at 1/500 dilution followed by <u>ab150120</u> (Alexa Fluor[®] 594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor® 488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.

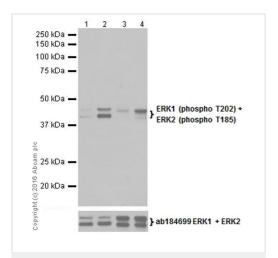


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR19401] (ab201015)

Immunohistochemical analysis of paraffin-embedded Human glioma tissue labeling ERK2 (phospho T185) with ab201015 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear with weak cytoplasm staining on Human glioma is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab97051</u> at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR19401] (ab201015)

All lanes: Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR19401] (ab201015) 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

Lane 3: Untreated PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

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

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 41 kDa

Observed band size: 42.44 kDa

Exposure time: 10 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The induction conditions refer to PMID:12454035; PMID:17026715;

PMID:22206868.

1 2 3
5ng
1ng
0.1ng
0.01ng

Dot Blot - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR19401] (ab201015)

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR19401] (ab201015)

Dot blot analysis of ERK2 (phospho T185) labeled with ab201015 at 1/1000 dilution.

Lane 1: ERK2 (pT185) phospho peptide: DHTGFLT(p)EYVATR aa179-191 peptide.

Lane 2: ERK2 Non-phospho peptide: DHTGFLTEYVATR aa179-191 peptide.

Lane 3: ERK2 (pY187) phospho peptide: DHTGFLTEY(p)VATR aa179-191 peptide.

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

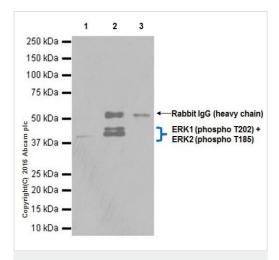
Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

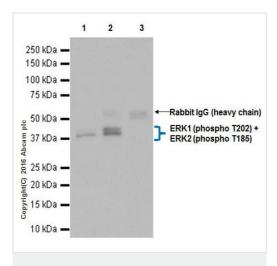
Immunohistochemical analysis of paraffin-embedded Human breast tissue labeling ERK2 (phospho T185) with ab201015 at 1/100 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on Human breast is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab97051</u> at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR19401] (ab201015)



Immunoprecipitation - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR19401] (ab201015)

ERK2 (phospho T185) was immunoprecipitated from 0.35 mg of NIH/3T3 (Mouse embryonic fibroblast cell line) treated with 50ng/ml PDGF for 40min whole cell lysate with ab201015 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab201015 at 1/1000 dilution. VeriBlot for IP Detection Reaction (HRP) (ab131366), was used for detection at 1/1000 dilution.

Lane 1: NIH/3T3 treated with 50ng/ml PDGF for 40min whole cell lysate, $10\mu g$ (lnput).

Lane 2: ab201015 IP in NIH/3T3 treated with 50ng/ml PDGF for 40min whole cell lysate.

Lane 3: Rabbit lgG,monoclonal [EPR25A]-lsotype
Control (<u>ab172730</u>) instead of ab201015 in NIH/3T3 treated with
50ng/ml PDGF for 40min whole cell lysate.

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

ERK2 (phospho T185) was immunoprecipitated from 0.35 mg of PC-12 (Rat adrenal gland pheochromocytoma cell line) treated with 100ng/ml NGF for 10min whole cell lysate with ab201015 at 1/30 dilution. Western blot was performed from the immunoprecipitate

using ab201015 at 1/1000 dilution. VeriBlot for IP Detection Reaction (HRP) (ab131366), was used for detection at 1/1000 dilution.

Lane 1: PC-12 treated with 100ng/ml NGF for 10min whole cell lysate, $10\mu g$ (lnput).

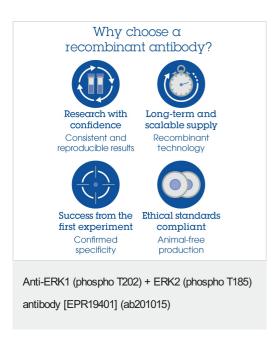
Lane 2: ab201015 IP in PC-12 treated with 100ng/ml NGF for 10min whole cell lysate.

Lane 3: Rabbit lgG,monoclonal [EPR25A]-lsotype
Control (ab172730) instead of ab201015 in PC-12 treated with
100ng/ml NGF for 10min whole cell lysate

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

Exposure time: 3 minutes.

Exposure time: 3 minutes.



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