abcam

Product datasheet

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

Recombinant RabMAb

3 References 14 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 within Human ERK1 aa 150-250 (phospho T202 + Y204). The exact sequence is proprietary. Database link: P27361
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 notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. This product is a recombinant rabbit monoclonal antibody.

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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR18444
Isotype	IgG

Applications

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

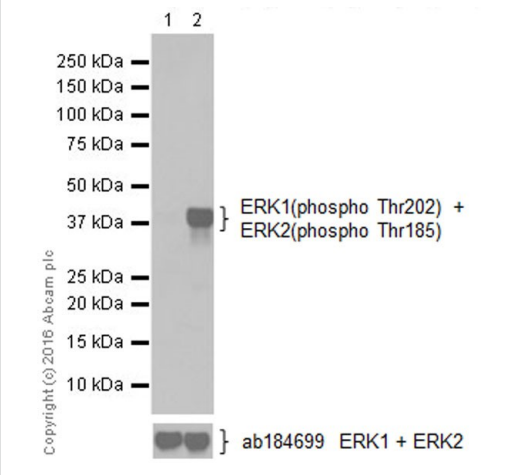
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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

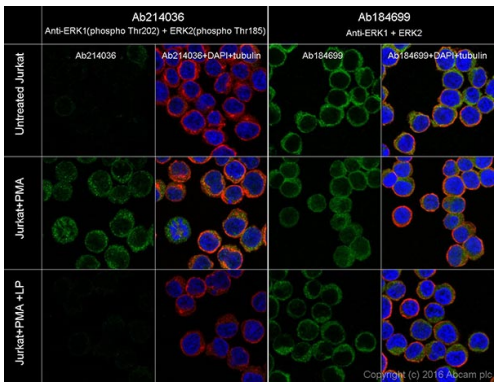
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 43, 41 kDa
Observed band size: 42,44 kDa
why is the actual band size different from the predicted?

Exposure time: 30 seconds

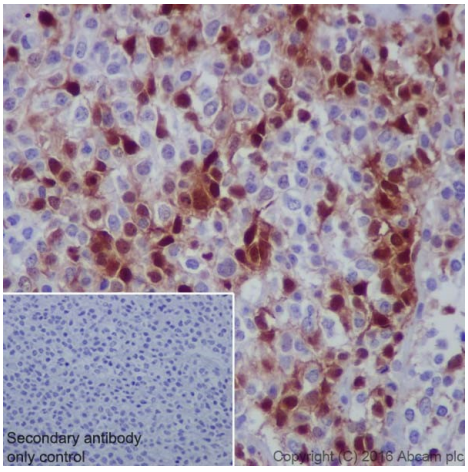
Blocking/Dilution buffer: 5% NFDM/TBST.



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

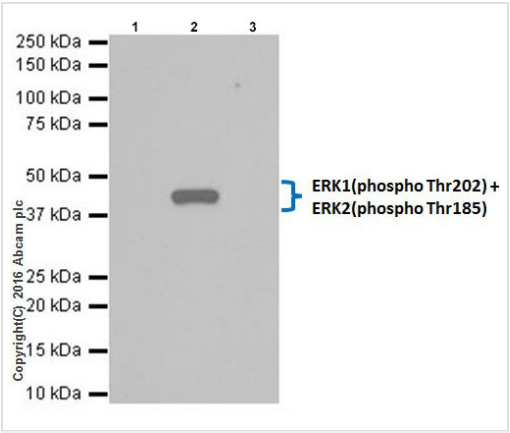
Immunohistochemical analysis of paraffin-embedded human glioma tissue labeling ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

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 IgG H&L (HRP) (ab97051) at 1/500 dilution.

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



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

ERK1 (phospho T202) + ERK2 (phospho T185) was immunoprecipitated from 0.35 mg of Jurkat (Human T cell leukemia cell line from peripheral blood) treated with 200 ng/ml PMA for 30 minutes 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) (ab131366), was used for detection at 1/10,000 dilution

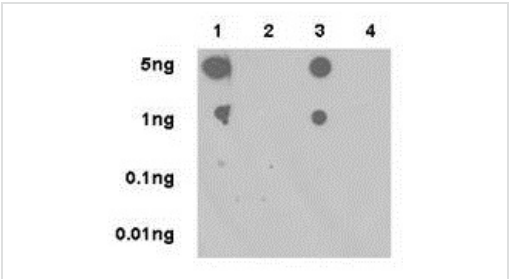
Lane 1: Jurkat treated with 200 ng/ml PMA for 30 minutes whole cell lysate 10µg (Input).

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.

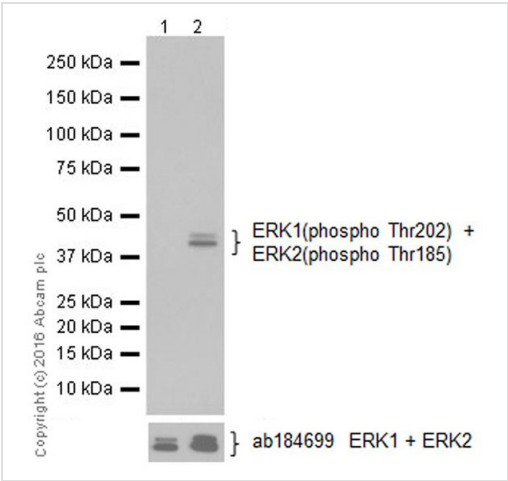
Exposure time: 3 minutes.



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

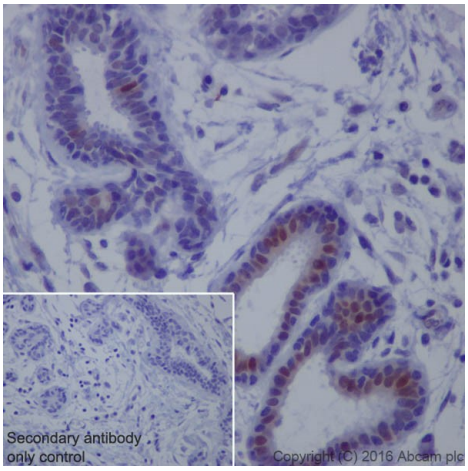
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 43, 41 kDa
Observed band size: 42,44 kDa why is the actual band size different from the predicted?

Exposure time: 2 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



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

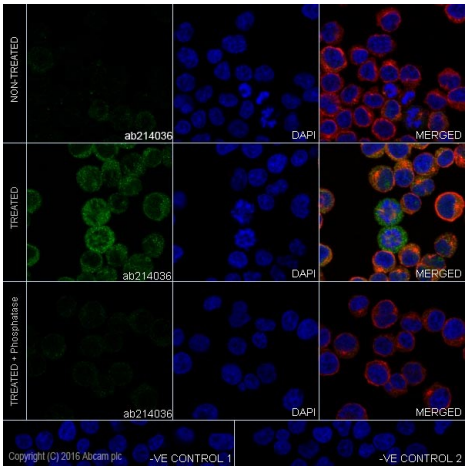
Immunohistochemical analysis of paraffin-embedded human breast tissue labeling ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

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 IgG H&L (HRP) (ab97051) at 1/500 dilution.

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



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.

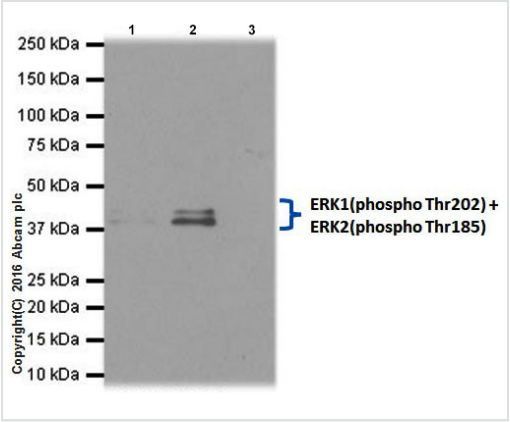
The nuclear counterstain is DAPI (blue).

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

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



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

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) (ab131366), was used for detection at 1/10,000 dilution

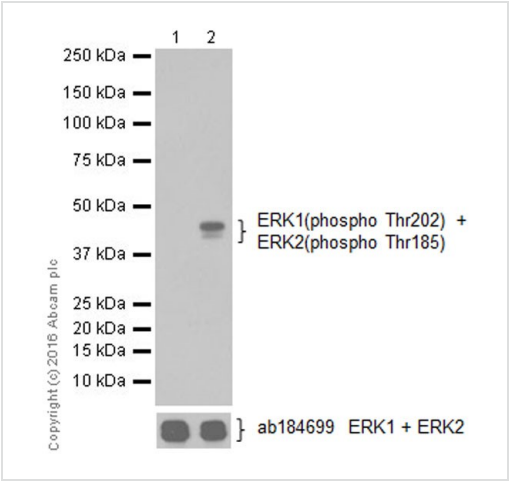
Lane 1: PC-12 treated with 200 ng/ml NGF for 4 days whole cell lysate 10µg (Input).

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

Lane 3: Rabbit IgG, 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.

Exposure time: 30 seconds.



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 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

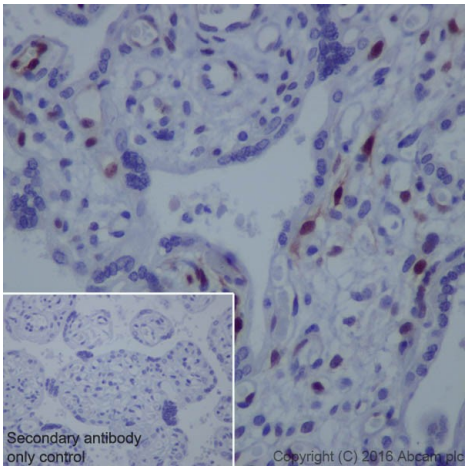
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 43, 41 kDa
Observed band size: 42,44 kDa why is the actual band size different from the predicted?

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



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

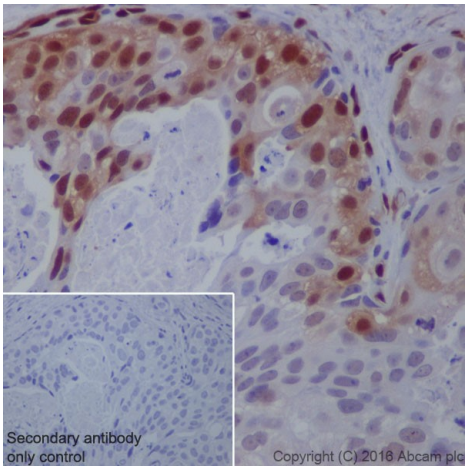
Immunohistochemical analysis of paraffin-embedded human placenta tissue labeling ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

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 IgG 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 paraffin-embedded sections) - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

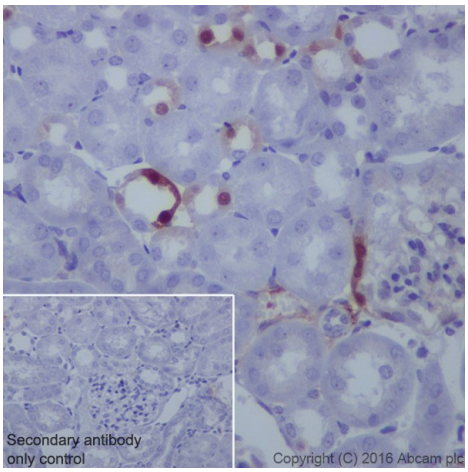
Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

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 IgG 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 paraffin-embedded sections) - Anti-ERK1 (phospho T202) + ERK2 (phospho T185) antibody [EPR18444] (ab214036)

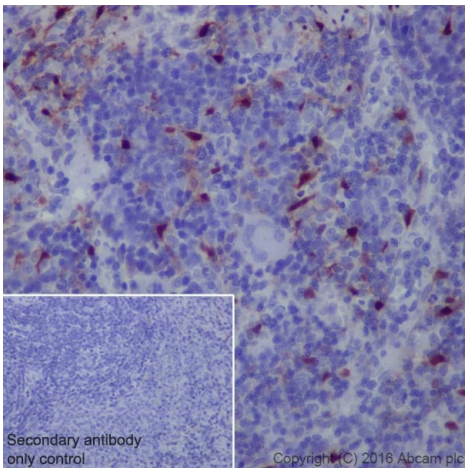
Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling ERK1 (phospho T202) + ERK2 (phospho T185) with ab214036 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

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 IgG 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 paraffin-embedded 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 IgG 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 IgG H&L (HRP) (ab97051) at 1/500 dilution.

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

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