abcam

Product datasheet

Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody ab4819

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Overview

Product name	Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody
Description	Rabbit polyclonal to Erk1 (pT202/pY204) + Erk2 (pT185/pY187)
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide corresponding to Human Erk1 (pT202/pY204) + Erk2 (pT185/pY187). This region is conserved among many species including rat, mouse, cow, frog, snail, nematode, and fruit fly. (Peptide available as **ab5313**, **ab5354**, **ab5255**)
Positive control	WB: MDA-MB-231, U-87 MG, Sh-SY5Y, HeLa, PC-12 whole cell lysates, MDA-MB-231 whole cell lysate with treatment of EGF(100 ng/mL for 15 mins. IHC-P: Human breast and colon carcinoma, mouse stomach tissue.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.30 Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol, 0.1% BSA BSA is IgG and protease free

Purity	Immunogen affinity purified
Purification notes	Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the sites of phosphorylation to remove antibody that is reactive with non-phosphorylated ERK 1 + 2. The final product is generated by affinity chromatography using an ERK 1 + 2-derived peptide that is phosphorylated at threonine 202/185 and tyrosine 204/187, respectively, within the activation loop.
Clonality	Polyclonal
Isotype	IgG

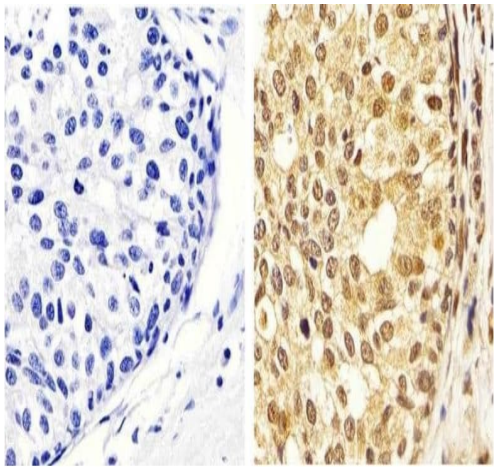
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab4819 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	★★★☆☆ (4)	1/1000. Predicted molecular weight: 44,42 kDa.
IHC-P		1/10 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

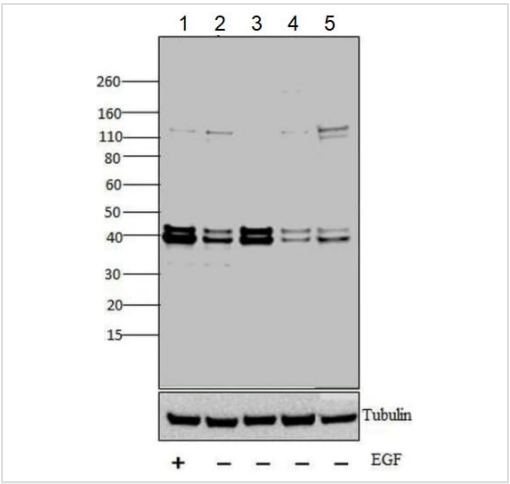
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody (ab4819)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue (right) labeling ERK1/2 (pTpY185/187) in the cytoplasm and nucleus with ab4819 at 1/50 dilution, compared to a negative control without primary antibody (left).

To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with ab4819 diluted in 3% BSA-PBS at a dilution of 1:50 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.



Western blot - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody (ab4819)

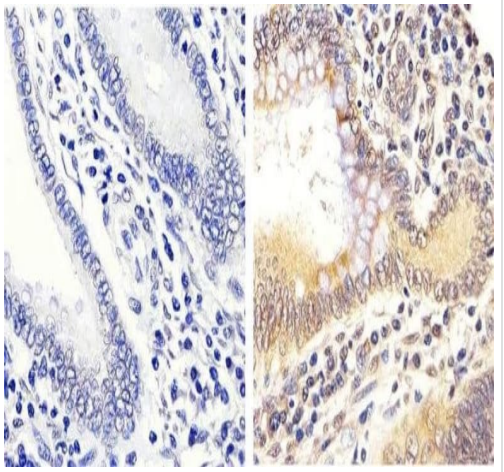
All lanes : Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody (ab4819) at 1/1000 dilution

Lane 1 : MDA-MB-231 (human breast adenocarcinoma cell line) whole cell lysate, with treatment of EGF(100 ng/mL for 15 mins)
Lane 2 : MDA-MB-231 whole cell lysate
Lane 3 : U-87 MG (human glioblastoma-astrocytoma epithelial cell line) whole cell lysate
Lane 4 : SH-SY5Y (human neuroblastoma cell line from bone marrow) whole cell lysate
Lane 5 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 30 µg per lane.

Predicted band size: 44,42 kDa

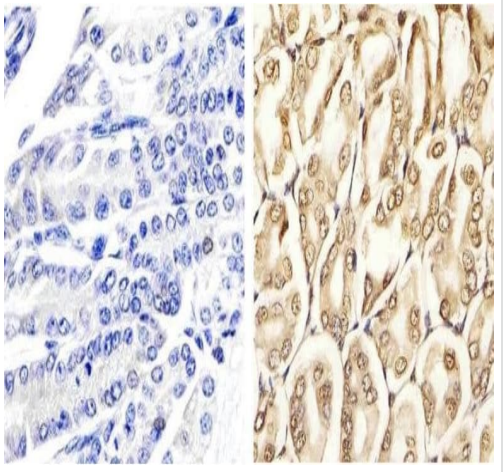
Bands of 42 kDa and 44 kDa corresponding to Phospho-p44 MAPK + p42 MAPK pThr185 + pTyr187 was observed across cell lines tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 12 % Bis-Tris gel, XCell SureLock™ Electrophoresis System and Novex® Sharp Pre-Stained Protein Standard. Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System. The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody (ab4819)

Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue (right) labeling ERK1/2 (pTpY185/187) in the cytoplasm and nucleus withab4819 at 1/20 dilution, compared to a negative control without primary antibody (left).

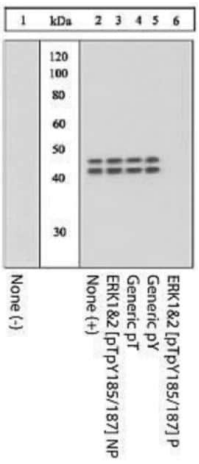
To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with ab4819 diluted in 3% BSA-PBS at a dilution of 1:20 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody (ab4819)

Immunohistochemical analysis of paraffin-embedded mouse stomach tissue (right) labeling ERK1/2 (pTpY185/187) in the cytoplasm and nucleus with ab4819 at 1/20 dilution, compared to a negative control without primary antibody (left).

To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with ab4819 diluted in 3% BSA-PBS at a dilution of 1/20 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.



Western blot - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody (ab4819)

All lanes : Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody (ab4819) at 1/1000 dilution

Lane 1 : PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate, unstimulated
Lanes 2-6 : PC-12 whole cell lysate, stimulated with 0.5 M sorbitol for 5 minutes

Secondary
All lanes : Goat F (ab')2 anti-rabbit IgG HRP conjugate

Predicted band size: 44,42 kDa

Extracts of PC12 cells were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF.

The membrane was blocked with a 5% BSA-TBST buffer overnight at 4°C, and then incubated with ab4819 for two hours at room temperature in a 3% BSA-TBST buffer, following its prior incubation with:

Lane 1 and 2: no peptide

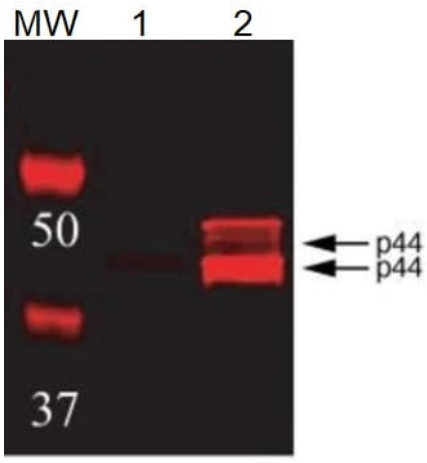
Lane 3: the non-phosphopeptide corresponding to the phosphopeptide immunogen

Lane 4: a generic phosphothreonine-containing peptide

Lane 5: a generic phosphotyrosine-containing peptide

Labe 6: the phosphopeptide immunogen

Detection: Pierce SuperSignal™ method.



Western blot - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody (ab4819)

All lanes : Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody (ab4819) at 1/1000 dilution

Lane 1 : NIH/3T3 (mouse embryonic fibroblast cell line) whole cell lysate
Lane 2 : NIH/3T3 whole cell lysate, treated with either PDGF

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Anti-rabbit secondary antibody conjugated to Alexa fluor 680

Predicted band size: 44,42 kDa

Data was analyzed on the LI-COR Odyssey® Infrared Imaging System.

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