# abcam

### Product datasheet

# Anti-ERK2 antibody [E460] - BSA and Azide free ab214170





RabMAb

# 3 References 7 Images

#### Overview

Product name Anti-ERK2 antibody [E460] - BSA and Azide free

**Description** Rabbit monoclonal [E460] to ERK2 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, ICC/IF, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human, Recombinant fragment

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

**Epitope** <u>ab32081</u> reacts with an epitope located in the C terminal region of ERK2.

Positive control WB: A431 whole cell lysate (ab7909), HeLa, HEK293, MES and PC12 whole cell lysates and

ERK2 recombinant protein. IHC-P: Rat pancreas tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa

cells.

**General notes** ab214170 is the carrier-free version of <u>ab32081</u>.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

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.

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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. Do Not Freeze.

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

**Clonality** Monoclonal

Clone number E460

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab214170 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
Flow Cyt (Intra)		Use at an assay dependent concentration. <b>ab199376</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 42 kDa (predicted molecular weight: 41 kDa). Can be blocked with <a href="mailto:ab205612">ab205612</a> .
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

# **Target**

#### **Function**

Involved in both the initiation and regulation of meiosis, mitosis, and postmitotic functions in differentiated cells by phosphorylating a number of transcription factors such as ELK1. Phosphorylates ElF4EBP1; required for initiation of translation. Phosphorylates microtubule-associated protein 2 (MAP2). Phosphorylates SPZ1 (By similarity). Phosphorylates heat shock factor protein 4 (HSF4) and ARHGEF2.

Acts as a transcriptional repressor. Binds to a [GC]AAA[GC] consensus sequence. Repress the expression of interferon gamma-induced genes. Seems to bind to the promoter of CCL5, DMP1, IFIH1, IFITM1, IRF7, IRF9, LAMP3, OAS1, OAS2, OAS3 and STAT1. Transcriptional activity is

independent of kinase activity.

Sequence similarities Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase

subfamily.

Contains 1 protein kinase domain.

**Domain** The TXY motif contains the threonine and tyrosine residues whose phosphorylation activates the

MAP kinases.

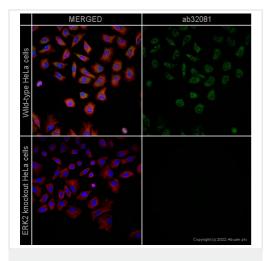
Post-translational modifications

Dually phosphorylated on Thr-185 and Tyr-187, which activates the enzyme. Dephosphorylated by

PTPRJ at Tyr-187.

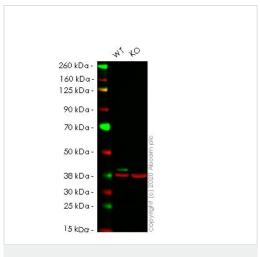
Cellular localization Nucleus.

#### **Images**



Immunocytochemistry/ Immunofluorescence - Anti-ERK2 antibody [E460] - BSA and Azide free (ab214170)

ab32081 staining ERK2 in wild-type HeLa cells (top panel) and ERK2 knockout HeLa cells (bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab32081 at 0.2µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), preadsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32081).



Western blot - Anti-ERK2 antibody [E460] - BSA and Azide free (ab214170)

All lanes: Anti-ERK2 antibody [E460] (ab32081) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: MAPK1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

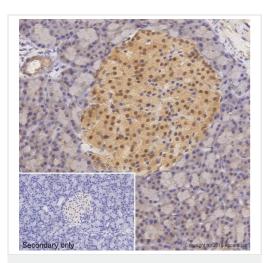
Performed under reducing conditions.

Predicted band size: 41 kDa Observed band size: 41 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab32081</u>).

**Lanes 1-2:** Merged signal (red and green). Green - <u>ab32081</u> observed at 41 kDa. Red - loading control <u>ab8245</u> observed at 37 kDa.

ab32081 Anti-ERK2 antibody [E460] was shown to specifically react with ERK2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265052 (knockout cell lysate ab257525) was used. Wild-type and ERK2 knockout samples were subjected to SDS-PAGE. ab32081 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 Dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

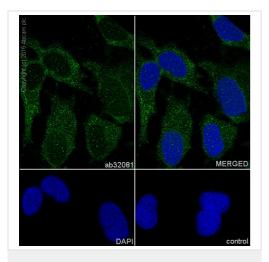


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ERK2 antibody [E460] - BSA and Azide free (ab214170)

IHC image of <u>ab32081</u> staining ERK2 in rat pancreas formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with <u>ab32081</u>, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset).

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32081).



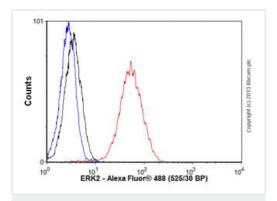
Immunocytochemistry/ Immunofluorescence - Anti-ERK2 antibody [E460] - BSA and Azide free (ab214170)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling ERK2 with <u>ab32081</u> at 1/400. Cells were fixed with 100% methanol. <u>ab150077</u>, an Alexa Fluor<sup>®</sup> 488-conjugated goat antirabbit lgG (1/1000) was used as the secondary antibody.

Control: PBS only.

Nuclear counter stain: DAPI.

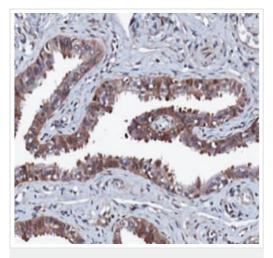
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32081</u>).



Flow Cytometry (Intracellular) - Anti-ERK2 antibody [E460] - BSA and Azide free (ab214170)

Overlay histogram showing HeLa cells stained with <u>ab32081</u> (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (<u>ab32081</u>, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was a goat anti-rabbit Alexa Fluor<sup>®</sup> 488 (lgG; H&L) (<u>ab150077</u>) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (0.1µg/1x10<sup>6</sup> cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32081).



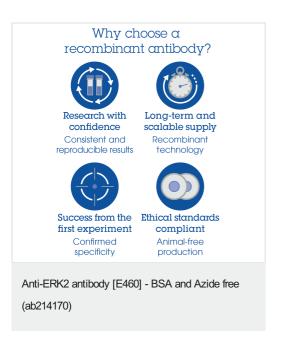
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ERK2 antibody [E460] - BSA and Azide free (ab214170)

Image from Emmanuel C et al. PLoS One. 2011 Mar 15;6(3):e17617. doi: 10.1371/journal.pone.0017617.; Fig 2.; March 15 2011 PLoS ONE 6(3): e17617.

Immunohistochemical analysis of Human fallopian tissue epithelium, staining ERK2 with <u>ab32081</u> at 1/50 dilution. Samples were incubated with primary antibody for 1 hour at room temperature. Staining was detected using DAB.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32081</u>).

Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.



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