Overview

Product name: Anti-ERK2 antibody [E460] ab32081

Description: Rabbit monoclonal [E460] to ERK2

Host species: Rabbit

Specificity: ab32081 recognises ERK2

Tested applications: Suitable for: IHC-P, WB, Flow Cyt, IP, ICC/IF

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

Immunogen: Synthetic peptide within Human ERK2 aa 300 to the C-terminus (C terminal). The exact sequence is proprietary.

Database link: P28482

Epitope: ab32081 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: HeLa cells.

General notes: A trial size is available to purchase for this antibody.

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 buffer: pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol, 0.05% BSA

Purity: Protein A purified

Clonality: Monoclonal

Clone number: E460
**Isotype**

IgG

**Applications**

Our **Abpromise guarantee** covers the use of ab32081 in the following tested applications.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1000 - 1/2000. Detects a band of approximately 42 kDa (predicted molecular weight: 41 kDa). Can be blocked with ab205612</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>1/80.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>1/400.</td>
</tr>
</tbody>
</table>

**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 EIF4EBP1; 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**
Western blot - Anti-ERK2 antibody [E460] (ab32081)

All lanes:

Lane 1: Wild-type HAP1 cell lysate
Lane 2: EKR2 knockout HAP1 cell lysate
Lane 3: NIH3T3 cell lysate
Lane 4: PC12 cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 41 kDa

ab32081 was shown to specifically react with ERK2 (MAPK1) in wild type HAP1 cells. No band was observed when ERK2 (MAPK1) knockout samples were used. Wild-type and ERK2 (MAPK1) knockout samples were subjected to SDS-PAGE. ab32081 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at a 1/1000 dilution and 1/20000 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 1/20000 dilution for 1 hour at room temperature before imaging.

IHC image of ab32081 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 ab32081, 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.
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling ERK2 with ab32081 at 1/400. Cells were fixed with 100% methanol. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody.
Control: PBS only.
Nuclear counter stain: DAPI.

**Lane 1**: Wild-type HAP1 cell lysate (20 µg)
**Lane 2**: ERK2 knockout HAP1 cell lysate (20 µg)
**Lanes 1 - 2**: Merged signal (red and green). Green - ab32081 observed at 44 kDa. Red signal from loading control - ab8226 observed at 42 kDa or ab8245, observed at 37 kDa.

This western blot image is a comparison between ab32081 and a competitor’s top cited rabbit polyclonal antibody.

Overlay histogram showing HeLa cells stained with ab32081 (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 (ab32081, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was a goat anti-rabbit Alexa Fluor® 488 (lgG; H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C.

Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10⁶ 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.
All lanes: Anti-ERK2 antibody [E460] (ab32081) at 1/1000 dilution

Lane 1: HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2: HEK293 (Human embryonic kidney cell line) Whole Cell Lysate
Lane 3: 46C (Mouse neural progenitor, selected for Sox1 expression cell line) Whole Cell Lysate
Lane 4: PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate
Lane 5: Recombinant Human ERK1 protein (ab43623) (ab43623)
Lane 6: Recombinant Human ERK2 protein (ab43625) (ab43625)

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) (ab175781) at 1/10000 dilution

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

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab32081 overnight at 4°C. Antibody binding was detected using ab175781 (goat anti-rabbit Alexa Fluor 790) at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.
Immunohistochemical analysis of Human fallopian tissue epithelium, staining ERK2 with ab32081 at 1/50 dilution. Samples were incubated with primary antibody for 1 hour at room temperature. Staining was detected using DAB.

**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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