

### Anti-ERK2 antibody [E460] ab32081

敲除验证
重组
RabMAb

★★★★★
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#### 概述

<b>产品名称</b>	Anti-ERK2抗体[E460]
<b>描述</b>	兔单克隆抗体[E460] to ERK2
<b>宿主</b>	Rabbit
<b>经测试应用</b>	<b>适用于:</b> IHC-P, Flow Cyt (Intra), WB, ICC/IF
<b>种属反应性</b>	<b>与反应:</b> Mouse, Rat, Human, Recombinant fragment
<b>免疫原</b>	Synthetic peptide within Human ERK2 aa 300 to the C-terminus (C terminal). The exact sequence is proprietary. Database link: <a href="#">P28482</a>
<b>表位</b>	ab32081 reacts with an epitope located in the C terminal region of ERK2.
<b>阳性对照</b>	WB: A431 whole cell lysate ( <a href="#">ab7909</a> ), 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.
<b>常规说明</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

#### 性能

<b>形式</b>	Liquid
<b>存放说明</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
<b>存储溶液</b>	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
<b>纯度</b>	Protein A purified

克隆	单克隆
克隆编号	E460
同种型	IgG

## 应用

**The Abpromise guarantee**      **Abpromise™**承诺保证使用ab32081于以下的经测试应用

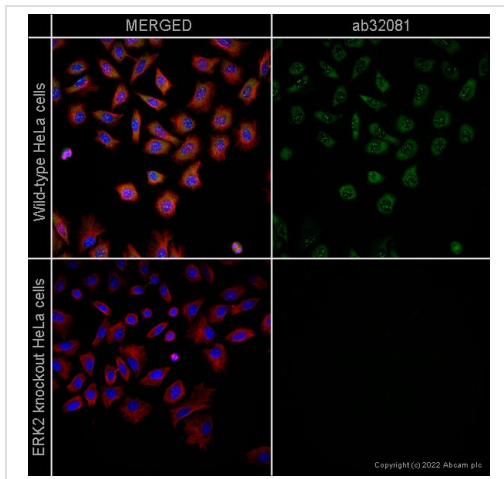
“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
IHC-P		Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/1000. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (1)	1/1000 - 1/2000. Detects a band of approximately 42 kDa (predicted molecular weight: 41 kDa). Can be blocked with <b>ab205612</b>
ICC/IF		Use a concentration of 0.2 µg/ml.

## 靶标

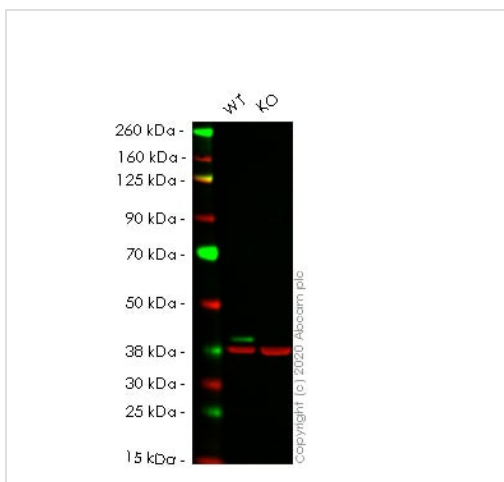
功能	<p>Involved in both the initiation and regulation of meiosis, mitosis, and postmitotic functions in differentiated cells by phosphorylating a number of transcription factors such as ELK1. Phosphorylates EIF4EBP1; required for initiation of translation. Phosphorylates microtubule-associated protein 2 (MAP2). Phosphorylates SPZ1 (By similarity). Phosphorylates heat shock factor protein 4 (HSF4) and ARHGEF2.</p> <p>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.</p>
序列相似性	<p>Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase subfamily.</p> <p>Contains 1 protein kinase domain.</p>
结构域	<p>The TXY motif contains the threonine and tyrosine residues whose phosphorylation activates the MAP kinases.</p>
翻译后修饰	<p>Dually phosphorylated on Thr-185 and Tyr-187, which activates the enzyme. Dephosphorylated by PTPRJ at Tyr-187.</p>
细胞定位	<p>Nucleus.</p>

## 图片



Immunocytochemistry/ Immunofluorescence - Anti-ERK2 antibody [E460] (ab32081)

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), pre-adsorbed 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.



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

**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.

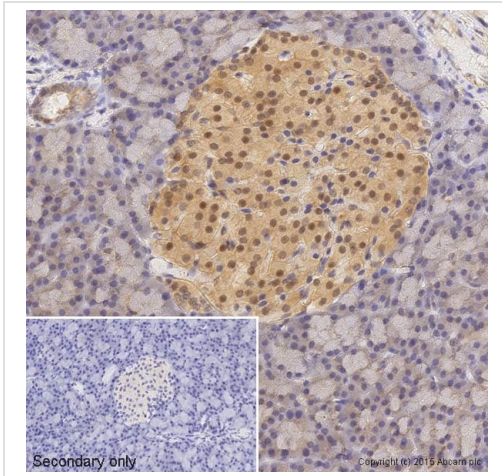
**Predicted band size:** 41 kDa

**Observed band size:** 41 kDa

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

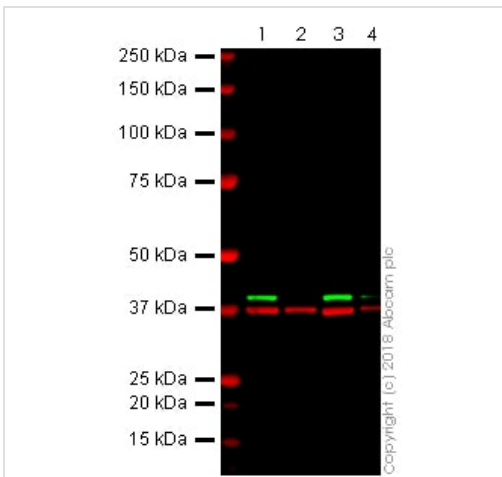
20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK2 antibody [E460] (ab32081)

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.



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

**All lanes :**

**Lane 1 :** Wild-type HAP1 cell lysate

**Lane 2 :** ERK2 knockout HAP1 cell lysate

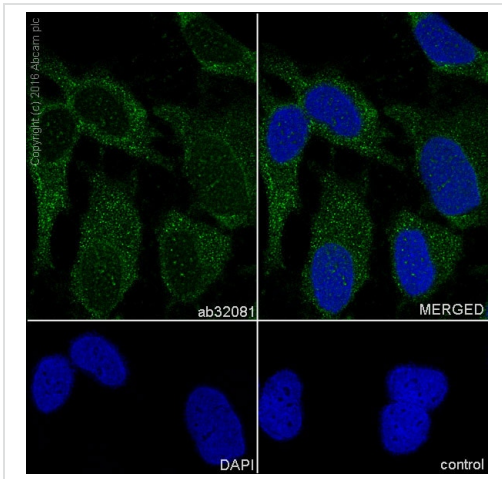
**Lane 3 :** NIH3T3 cell lysate

**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.

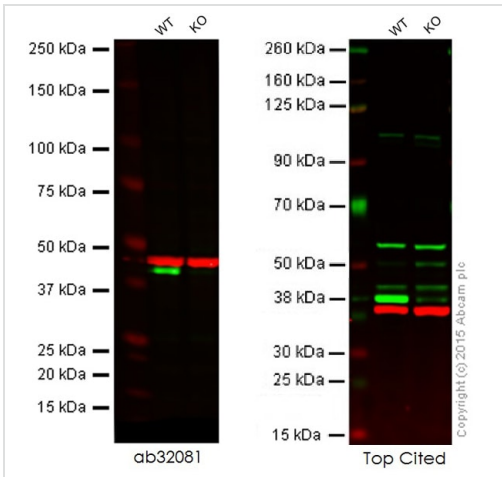


Immunocytochemistry/ Immunofluorescence - Anti-ERK2 antibody [E460] (ab32081)

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.



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

**All lanes** : Anti-ERK2 antibody [E460] (ab32081)

**Lane 1** : Wild-type HAP1 cell lysate

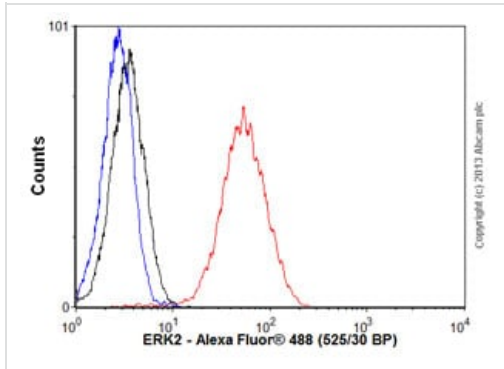
**Lane 2** : ERK2 knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size:** 41 kDa

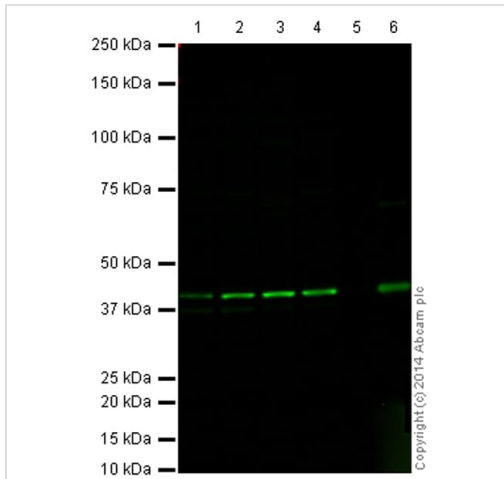
**Lanes 1 - 2:** Merged signal (red and green). Green - ab32081 observed at 44 kDa. Red signal from loading control - **ab8226** observed at 42 kDa or **ab8245**, observed at 37 kDa.

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



Flow Cytometry (Intracellular) - Anti-ERK2 antibody [E460] (ab32081)

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 (IgG; H&L) ([ab150077](#)) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (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.



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

**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](#))

Lysates/proteins at 20 µg per lane.

### Secondary

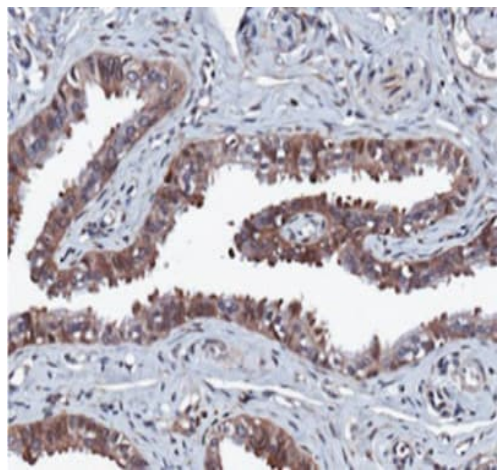
**All lanes :** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) ([ab175781](#)) at 1/10000 dilution

**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.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK2 antibody [E460] (ab32081)

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.

### Why choose a recombinant antibody?

 <b>Research with confidence</b> Consistent and reproducible results	 <b>Long-term and scalable supply</b> Recombinant technology
 <b>Success from the first experiment</b> Confirmed specificity	 <b>Ethical standards compliant</b> Animal-free production

Anti-ERK2 antibody [E460] (ab32081)

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