abcam

Product datasheet

Anti-ERK1 antibody [Y72] ab32537

敲除验证 重组 RabMAb

4 Abreviews 52 References 20 图像

概述

产品名称	Anti-ERK1抗体[Y72]
描述	兔单克隆抗体[Y72] to ERK1
宿主	Rabbit
特异性	This antibody recognises ERK1.The antibody does not cross-react with other MAP kinases.
经测试应用	**适用于:** WB, IP, IHC-P, Flow Cyt (Intra), ICC/IF
种属反应性	**与反应:** Mouse, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
表位	ab32537 reacts with an epitope located in the N terminal region of ERK1.
阳性对照	WB: HeLa, HEK-293T, HEK293, Jurkat and RAW264.7, Mouse brain, heart, kidney, spleen and NIH/3T3 whole cell lysates and ERK1 recombinant protein. IHC: Human lung carcinoma, human cervix carcinoma and human tonsil tissues. ICC/IF: Wild-type HAP1 and Jurkat cells. Flow Cyt (intra): HeLa and Jurkat cells. HAP1 cells. IP: Jurkat cells.
常规说明	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**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	Y72
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab32537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。

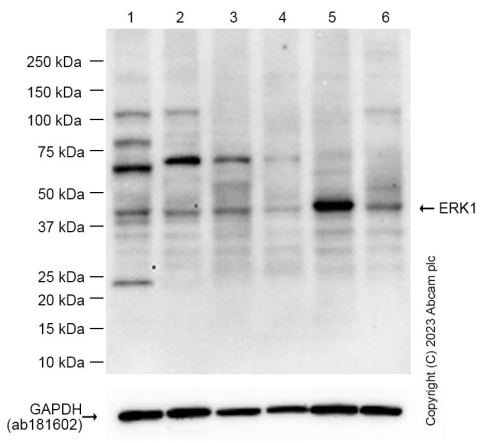
应用	Ab评论	说明
WB	★★★★★ (1)	1/1000. Detects a band of approximately 44 kDa (predicted molecular weight: 43 kDa).
IP		1/20 - 1/40.
IHC-P		Use a concentration of 2 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
Flow Cyt (Intra)		1/30 - 1/40. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (2)	1/400. This product gave a positive signal in HAP1 cells fixed with 4% formaldehyde (10 min) and 100% methanol (5 min).

靶标

功能	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 ELK-1. Phosphorylates EIF4EBP1; required for initiation of translation. Phosphorylates microtubule-associated protein 2 (MAP2). Phosphorylates SPZ1 (By similarity). Phosphorylates heat shock factor protein 4 (HSF4).
序列相似性	Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase subfamily. Contains 1 protein kinase domain.
结构域	The TXY motif contains the threonine and tyrosine residues whose phosphorylation activates the MAP kinases.
翻译后修饰	Dually phosphorylated on Thr-202 and Tyr-204, which activates the enzyme. Dephosphorylated by PTPRJ at Tyr-204.

图片

图片



Western blot - Anti-ERK1 antibody [Y72] (ab32537)

All lanes : Anti-ERK1 antibody [Y72] (ab32537) at 1/1000 dilution

Lane 1 : Mouse brain lysate
Lane 2 : Mouse heart lysate
Lane 3 : Mouse kidney lysate
Lane 4 : Mouse spleen lysate
Lane 5 : Raw 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 6 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 43 kDa
Observed band size: 43 kDa

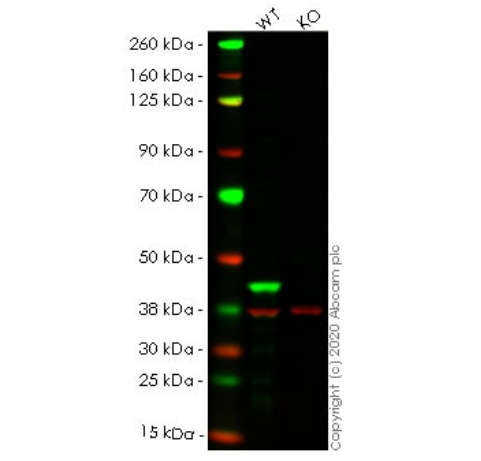
Exposure time: 180 seconds

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

This blot was developed using a **high** sensitivity ECL substrate.

ab181602 was used as a loading control.



Western blot - Anti-ERK1 antibody [Y72] (ab32537)

All lanes : Anti-ERK1 antibody [Y72] (ab32537) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : MAPK3 knockout HEK-293T cell lysate

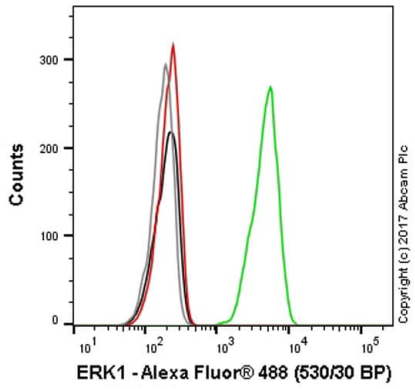
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 43 kDa
Observed band size: 43 kDa

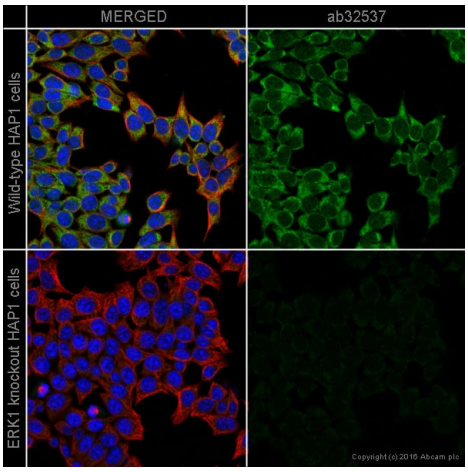
Lanes 1- 2: Merged signal (red and green). Green - ab32537 observed at 43 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab32537 was shown to react with ERK1 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266519** (knockout cell lysate **ab257099**) was used. Wild-type HEK-293T and MAPK3 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab32537 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 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.



Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] (ab32537)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-MAPK3 knockout cells (red line) stained with ab32537. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab32537, 1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150081**) at 1/2000 dilution for 30 min at 22°C. A Rabbit IgG isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-MAPK3 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

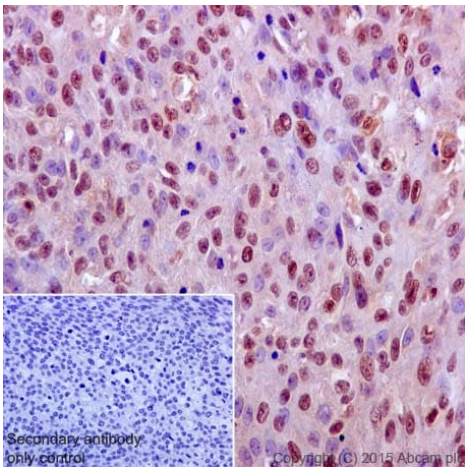


Immunocytochemistry/ Immunofluorescence - Anti-ERK1 antibody [Y72] (ab32537)

ab32537 staining ERK1 in wild-type HAP1 cells (top panel) and ERK1 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 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 with ab32537 at 1/400 dilution and **ab7291** at 1ug/ml concentration overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to Mouse IgG (Alexa Fluor® 594) (**ab150117**) at 2ug/ml (shown in pseudo-color red). Nuclear DNA was labelled in blue with DAPI.

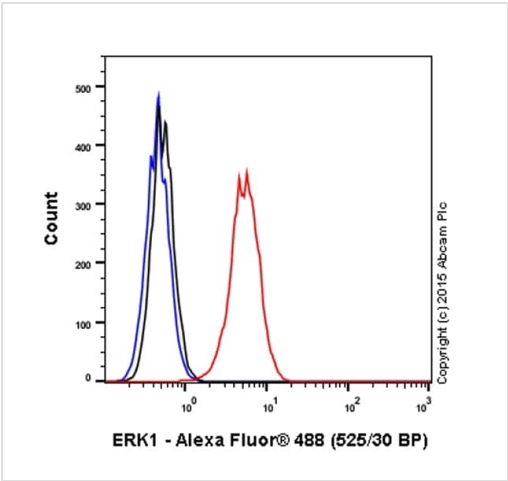
This product also gave a positive signal under the same testing conditions in HAP1 cells fixed with 4% formaldehyde (10 min).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK1 antibody [Y72] (ab32537)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labelling ERK1 with purified ab32537 at a dilution of 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

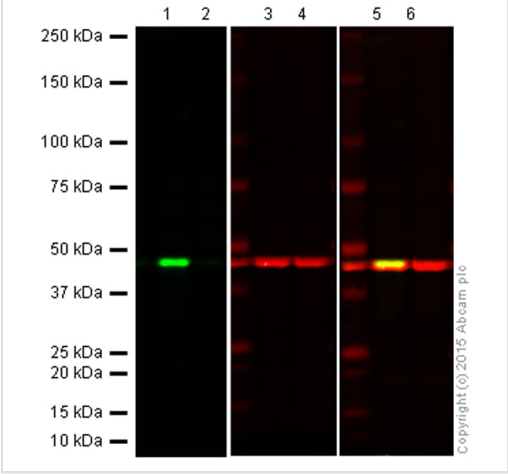


Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] (ab32537)

Overlay histogram showing HeLa cells stained with ab32537 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween 20 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 (ab32537, 1/11312) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150081**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (**ab172730**, 0.01µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control.

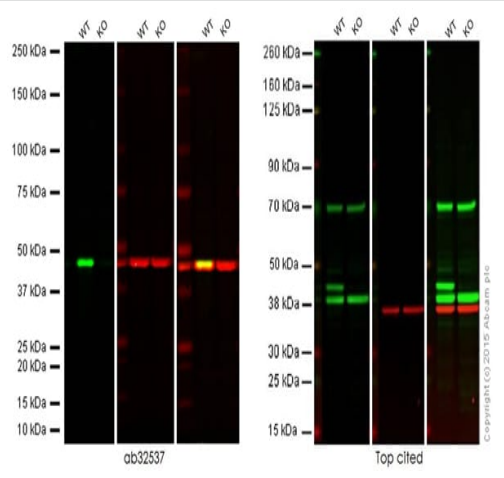
Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

This antibody gave a positive signal in HeLa cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween 20 for 20 min used under the same conditions.



Western blot - Anti-ERK1 antibody [Y72] (ab32537)

Lanes 1, 3 and 5: Wild-type HAP1 cell lysate (20 µg)
Lanes 2, 4 and 6: ERK1 knockout HAP1 cell lysate (20 µg)
Lanes 1 and 2: Green signal from target - ab32537 observed at 42 kDa
Lanes 3 and 4: Red signal from loading control - **ab8226** observed at 42 kDa
Lanes 5 and 6: Merged (red and green) signal
ab32537 was shown to specifically react with ERK1 when ERK1 knockout samples were used.
Wild-type and ERK1 knockout samples were subjected to SDS-PAGE. ab32537 and **ab8226** (loading control to beta actin) were both diluted 1/1000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) andGoat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.

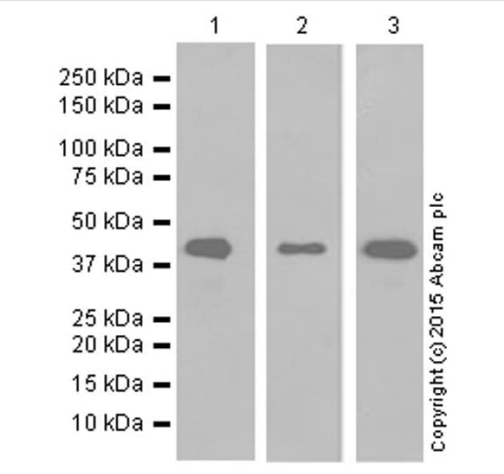


Western blot - Anti-ERK1 antibody [Y72] (ab32537)

Lanes 1, 3 and 5: Wild-type HAP1 cell lysate (20 µg)
Lanes 2, 4 and 6: ERK1 knockout HAP1 cell lysate (20 µg)
Lanes 1 and 2: Green signal from target
Lanes 3 and 4: Red signal from loading control
Lanes 5 and 6: Merged (red and green) signal

Red - loading control, **ab8226** observed at 42 kDa or **ab8245**, observed at 37 kDa

This western blot image is a comparison between ab32537 and a competitor's top cited rabbit polyclonal antibody.



Western blot - Anti-ERK1 antibody [Y72] (ab32537)

All lanes : Anti-ERK1 antibody [Y72] (ab32537) at 1/10000 dilution (purified)

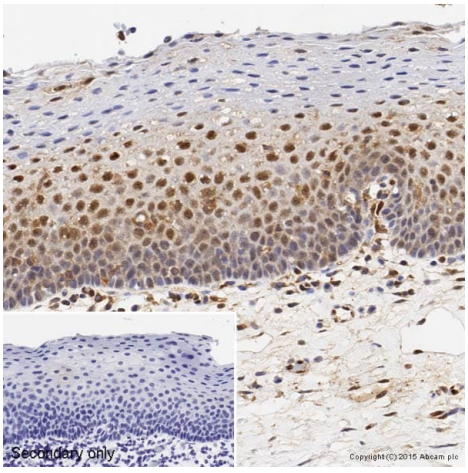
Lane 1 : 293T whole cell lysate
Lane 2 : HeLa whole cell lysate
Lane 3 : Jurkat whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

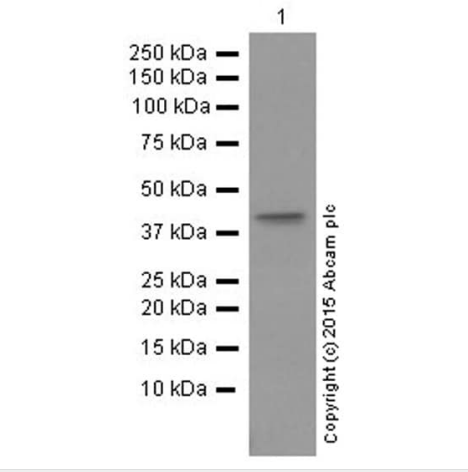
Predicted band size: 43 kDa
Observed band size: 44 kDa

Blocking and dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK1 antibody [Y72] (ab32537)

IHC image of ab32537 staining ERK1 in Human tonsil 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 ab32537, 2µg/ml dilution, 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.



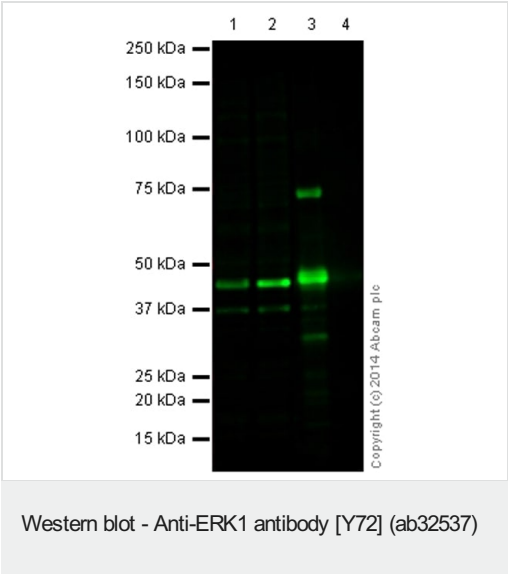
Western blot - Anti-ERK1 antibody [Y72] (ab32537)

Anti-ERK1 antibody [Y72] (ab32537) at 1/1000 dilution (purified) + RAW264.7 whole cell lysate at 20 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

Predicted band size: 43 kDa
Observed band size: 44 kDa

Blocking and dilution buffer: 5% NFDM/TBST



Western blot - Anti-ERK1 antibody [Y72] (ab32537)

All lanes : Anti-ERK1 antibody [Y72] (ab32537) at 1/1000 dilution

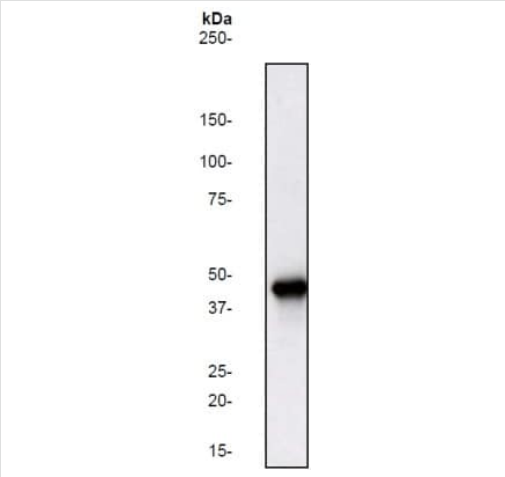
Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate
Lane 3 : Recombinant Human ERK1 protein (**ab43623**) (**ab43623**)
Lane 4 : Recombinant Human ERK2 protein (**ab43625**)

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: 43 kDa
Observed band size: 44 kDa

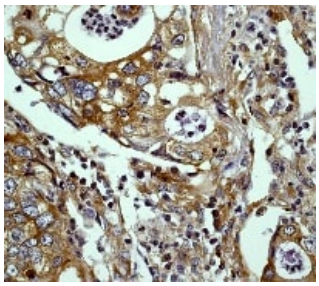
This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab32537 overnight at 4°C. Antibody binding was detected using a goat **anti-rabbit Alexa Fluor® 790**) **ab175781** at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.



Western blot - Anti-ERK1 antibody [Y72] (ab32537)

Anti-ERK1 antibody [Y72] (ab32537) at 1/1000 dilution (unpurified) + Jurkat cell lysate

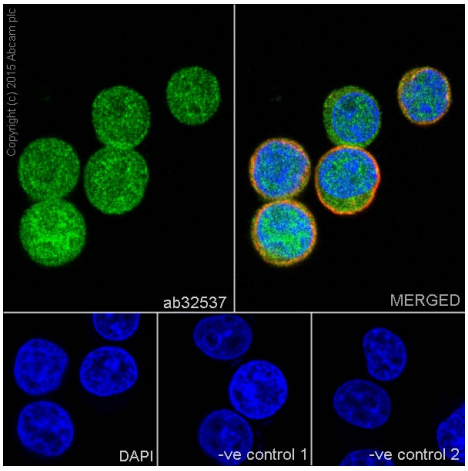
Predicted band size: 43 kDa
Observed band size: 43 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK1 antibody [Y72] (ab32537)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung carcinoma tissue labelling ERK1 with unpurified ab32537.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

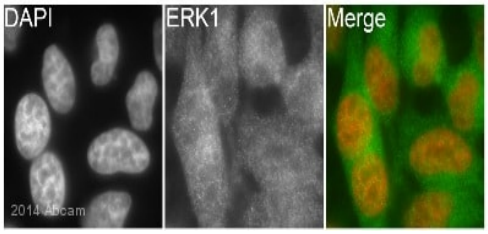


Immunocytochemistry/ Immunofluorescence - Anti-ERK1 antibody [Y72] (ab32537)

Immunocytochemistry/Immunofluorescence analysis of Jurkat cells labelling ERK1 with purified ab32537 at a dilution of 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

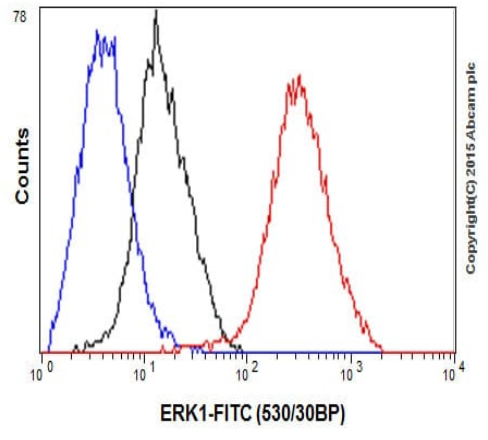
Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).



Immunocytochemistry/ Immunofluorescence - Anti-ERK1 antibody [Y72] (ab32537)

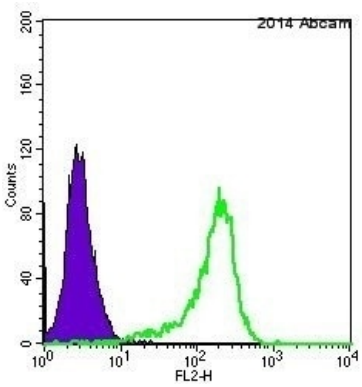
This image is courtesy of an Abreview submitted by Kirk McManus

Unpurified ab32537 staining ERK1 in HeLa cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton X-100 in PBS. Samples were incubated with primary antibody (1/500 in PBS) for 1 hour at 22°C. **ab150081**, a goat **anti rabbit Alexa Fluor® 488** (1/200) was used as the secondary antibody. Counterstained with DAPI. Cytoplasmic and nuclear staining shown.



Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] (ab32537)

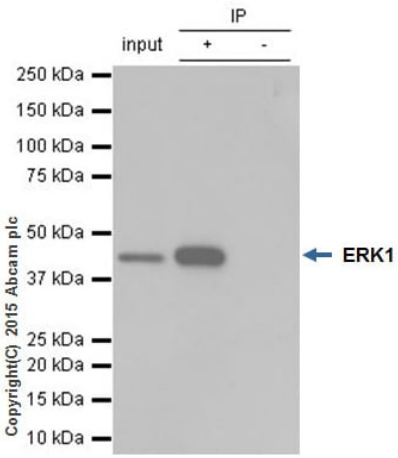
Intracellular Flow Cytometry analysis ofJurkat cells labelling ERK1 with purified ab32537 at a dilution of 1/30 (red). Cells were fixed with 4% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] (ab32537)

This image is courtesy of an anonymous Abreview

Unpurified ab32537 staining ERK1 (green) in HEK293 cells by intracellular flow cytometry. Cells were fixed with paraformaldehyde and permeabilized with 70% methanol. The sample was incubated with the primary antibody (1/40 in PBS + 0.2% BSA + 0.1% sodium azide) for 1 hour at 22°C. A phycoerythrin-conjugated goat anti-rabbit IgG (1/100) was used as the secondary antibody. Gating Strategy: Live Cells. Purple plot represents isotype control.



Immunoprecipitation - Anti-ERK1 antibody [Y72] (ab32537)

ab32537 (purified) at a dilution of 1/20 immunoprecipitating ERK1 in Jurkat whole cell lysate.

Lane 1 (input): Jurkat whole cell lysate (10µg)

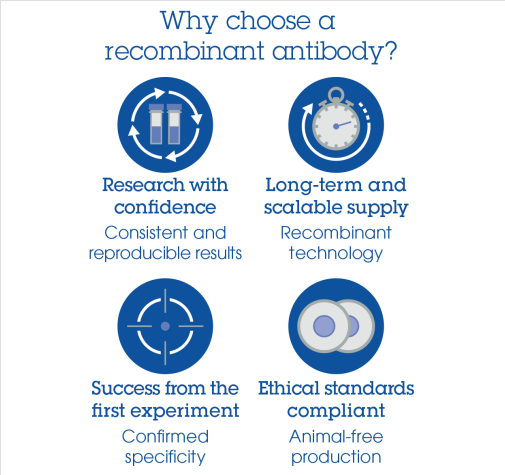
Lane 2 (+): ab32537 + Jurkat whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32537 in Jurkat whole cell lysate.

For western blotting, **ab131366** VeriBlot for IP (HRP) was used for detection (1/10000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Anti-ERK1 antibody [Y72] (ab32537)

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