abcam

Product datasheet

Anti-ERK1 antibody [Y72] - BSA and Azide free ab214168

KO VALIDATED Recombinant RabMAb

5 References 17 Images

Overview

Product name	Anti-ERK1 antibody [Y72] - BSA and Azide free
Description	Rabbit monoclonal [Y72] to ERK1 - BSA and Azide free
Host species	Rabbit
Specificity	This antibody recognises ERK1.The antibody does not cross-react with other MAP kinases.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Epitope	ab214168 reacts with an epitope located in the N terminal region of ERK1.
Positive control	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.
General notes	ab214168 is the carrier-free version of **ab32537**. Our **carrier-free** 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 **conjugation kits** 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® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® 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**.

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.

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	Y72
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab214168 in the following tested applications.

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

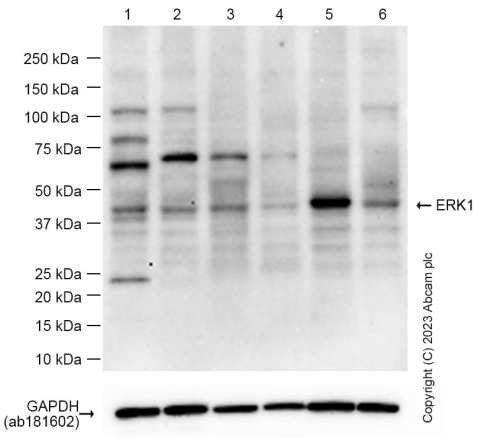
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control.
WB		Use at an assay dependent concentration. Detects a band of approximately 44 kDa (predicted molecular weight: 43 kDa). Can be blocked with **ab204281**.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		Use at an assay dependent concentration.

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 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).
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-202 and Tyr-204, which activates the enzyme. Dephosphorylated by PTPRJ at Tyr-204.

Images



Western blot - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

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

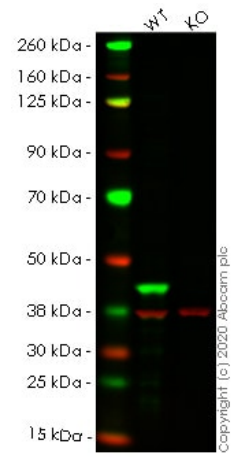
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

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

ab181602 was used as a loading control.

This data was developed using the same antibody clone in a different buffer formulation (**ab32537**).



Western blot - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

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.

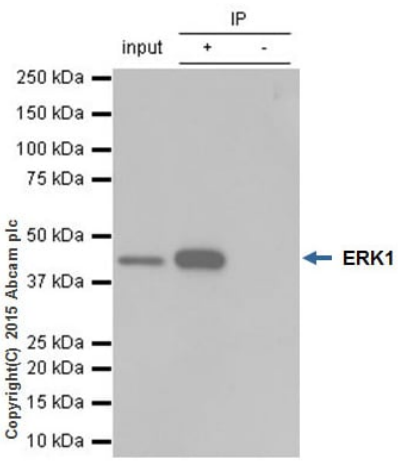
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab32537**).

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.



Immunoprecipitation - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

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.

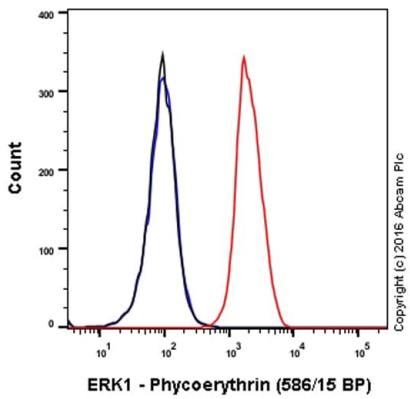
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab32537** in Jurkat whole cell lysate.

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution.

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32537**).



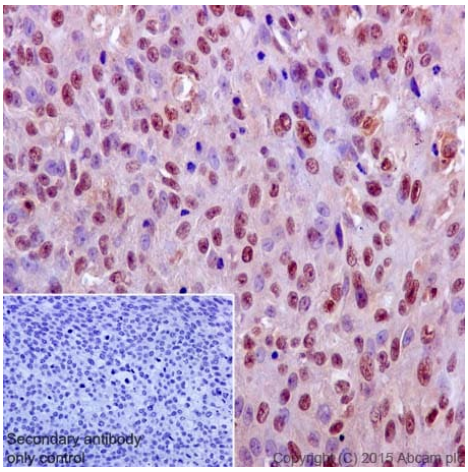
Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

Clone Y72 (ab214168) has been successfully conjugated by Abcam. This image was generated using Anti-ERK1 antibody [Y72] (PE). Please refer to **ab210828** for protocol details.

Overlay histogram showing Jurkat cells stained with **ab210828** (red line). The cells were fixed with 4% formaldehyde and then permeabilized with 90% methanol at -20°C 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 (**ab210828**, 1/5000 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was rabbit IgG (monoclonal) Phycoerythrin (**ab209478**) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

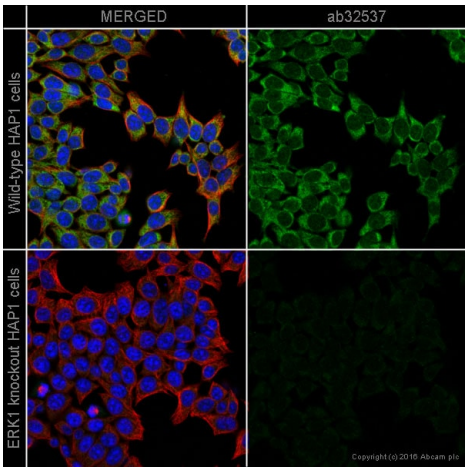
Acquisition of >5,000 events were collected using a 50mW Yellow/Green laser (561nm) and 586/15 bandpass filter.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32537**).



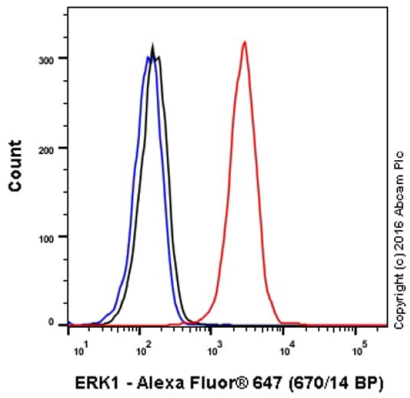
Immunocytochemistry/ Immunofluorescence - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32537**).



Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

Clone Y72 (ab214168) has been successfully conjugated by Abcam. This image was generated using Anti-ERK1 antibody [Y72] (Alexa Fluor® 647). Please refer to **ab190579** for protocol details.

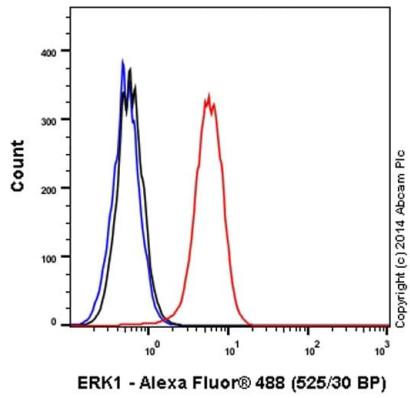
Overlay histogram showing HeLa cells stained with **ab190579** (red line). The cells were fixed with 4% formaldehyde and then permeabilized with 0.1% PBS-Triton X-100 for 15 min.

The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (**ab190579**, 1/5000 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Alexa Fluor® 647 (**ab199093**) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 40 mW Red laser (640nm) and 670/14 bandpass filter.

This antibody gave a positive signal in HeLa cells fixed with 80% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

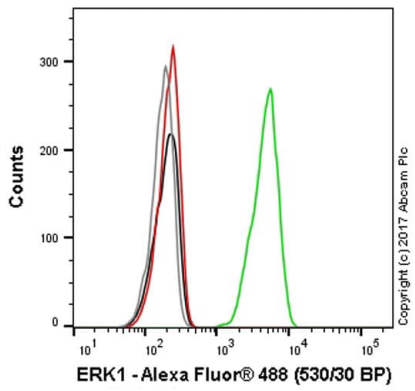
Clone Y72 (ab214168) has been successfully conjugated by Abcam. This image was generated using Anti-ERK1 antibody [Y72] (Alexa Fluor® 488). Please refer to **ab190200** for protocol details.

Overlay histogram showing HeLa cells stained with **ab190200** (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 (**ab190200**, 1/500 dilution) for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) Alexa Fluor® 488 used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon

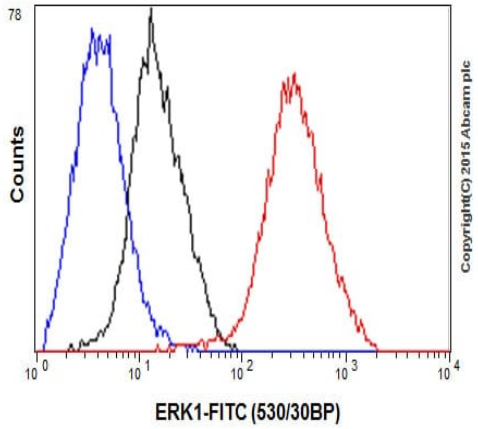
ion laser (488nm) and 525/30 bandpass filter.

This antibody gave a positive signal in HeLa fixed with 4% formaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

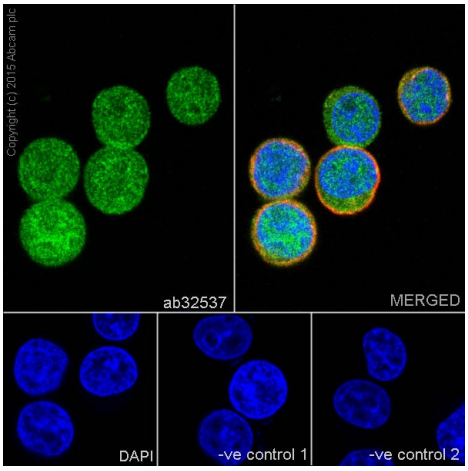
Overlay histogram showing HAP1 wildtype (green line) and HAP1-MAPK3 knockout cells (red line) stained with **ab32537**. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block 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. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32537**).



Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

Intracellular Flow Cytometry analysis of Jurkat 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32537**).



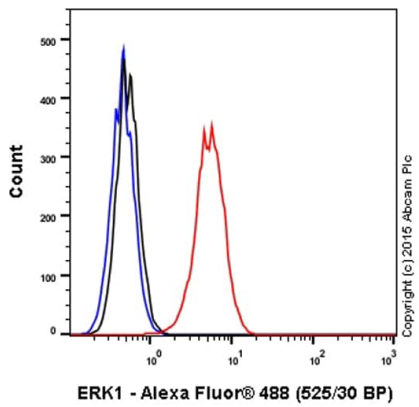
Immunocytochemistry/ Immunofluorescence - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32537**).



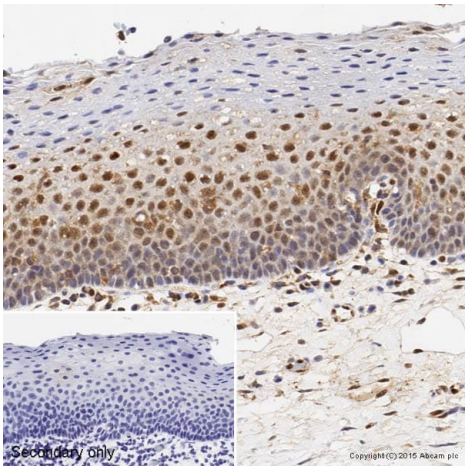
Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

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.

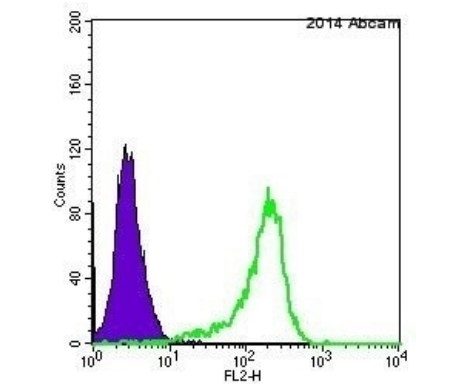
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32537**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32537**).

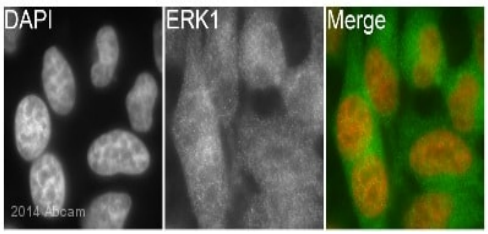


Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32537**).

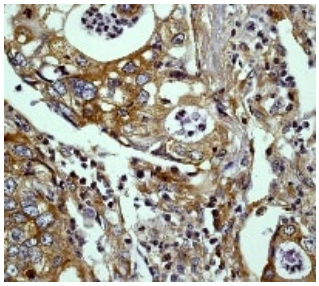


Immunocytochemistry/ Immunofluorescence - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32537**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32537**).

Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.

Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

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