# abcam

## Product datasheet

# Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [E337] ab32538

Recombinant RabMAb

29 References 6 Images

Overview

Product name Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [E337]

Description Rabbit monoclonal [E337] to Erk1 (pT202/pY204) + Erk2 (pT185/pY187)

Host species Rabbit

**Specificity** The antibody detects ERK1 phosphorylated on Threonine 202 and Tyrosine 204 and ERK2

phosphorylated on Threonine 185 and Tyrosine 187.

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

Unsuitable for: IP

Species reactivity Reacts with: Human

Immunogen Synthetic peptide within Human Erk1 (pT202/pY204) + Erk2 (pT185/pY187). The exact sequence

is proprietary.

(Peptide available as ab205613)

Positive control WB: Serum starved A431 cell lysate treated with EGF. IHC-P: Human thyroid gland cancer tissue.

ICC/IF: A431 cells +- EGF.

**General notes**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<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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

**Properties** 

Form Liquid

**Storage instructions** Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

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Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 50% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

**Clonality** Monoclonal

Clone number E337 lsotype lgG

#### **Applications**

The Abpromise guarantee Our Abpromise gu

Our  $\underline{\textbf{Abpromise guarantee}}$  covers the use of ab32538 in the following tested applications.

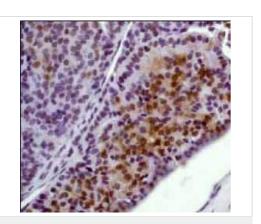
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/500 - 1/10000. Detects a band of approximately 42, 44 kDa (predicted molecular weight: 42 , 44 kDa).
IHC-P		1/50. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/1000.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/200 - 1/250.

**Application notes** 

Is unsuitable for IP.

#### **Images**

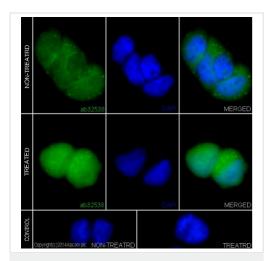


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [E337] (ab32538)

Immunohistochemical analysis of paraffin-embedded human thyroid gland cancer using anti-ERK1(pT202/pY204)/ERK2(pT185/pY187) (ab32538) at dilution of 1:50.

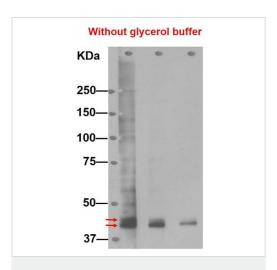
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

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



Immunocytochemistry/ Immunofluorescence - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [E337] (ab32538)

Immunocytochemistry/Immunofluorescence analysis of 4% paraformaldehyde A431+-EGF(100ng/ml,5min) labelling Erk1 (pT202/pY204) + Erk2 (pT185/pY187) with ab32538 at dilution of 1/200. The secondary antibody used was Alexa Fluor® 488 Goat-Anti-Rabbit lgG (ab150077) at dilution of 1/400. The counter stain was done with DAPI (blue).



Western blot - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [E337] (ab32538)

**Lane 1**: Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [E337] (ab32538) at 1/500 dilution

**Lane 2**: Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [E337] (ab32538) at 1/2000 dilution

**Lane 3**: Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [E337] (ab32538) at 1/10000 dilution

All lanes: A431 treated with EGF for 10 minutes

Lysates/proteins at 0.1 µg/ml per lane.

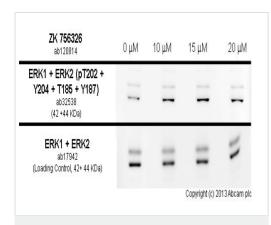
### **Secondary**

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

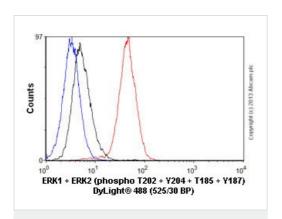
Predicted band size: 42,44 kDa Observed band size: 42.44 kDa

Exposure time: 3 minutes

First-antibody diluted with 1% BSA.



Western blot - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [E337] (ab32538)

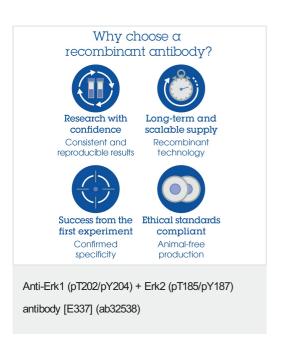


Flow Cytometry (Intracellular) - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [E337] (ab32538)

THP1 cells were incubated at 37°C for 3 minutes with vehicle control (0  $\mu$ M) and different concentrations of ZK 756326 (ab120814). Increased expression of ERK1 (phospho T202 + Y204 ) + ERK2 (phospho T185 + Y187) (ab32538) in THP1 cells correlates with an increase in ZK 756326 concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 10  $\mu$ g of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated with ab32538 at 1/500 dilution and ab17942 at 1  $\mu$ g /ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (ab97051) at 1/10000 dilution and visualised using ECL development solution.

Overlay histogram showing HeLa cells stained with ab32538 (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 (ab32538, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was a goat anti-rabbit DyLight® 488 (lgG; H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1 $\mu$ g/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.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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