abcam

Product datasheet

Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] ab76299

Recombinant RabMAb

79 References 10 Images

Overview

Product name Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y]

Description Rabbit monoclonal [EP197Y] to Erk1 (pT202/pY204) + Erk2 (pT185/pY187)

Host species Rabbit

Specificity This antibody detects Erk1 phosphorylated at Threonine 202 and Tyrosine 204 and Erk2

phosphorylated at Threonine 185 and Tyrosine 187.

Tested applications Suitable for: WB, IP, Dot blot, ELISA

Unsuitable for: Flow Cyt,ICC/IF or IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: A431 (treated with EGF), SH-SY5Y (treated with NGF), PC-12 (treated with NGF), HeLa and

NIH/3T3 whole cell lysates. IP: A431 (treated with EGF) and PC-12 (treated with NGF) whole cell

lysates.

General notesThis 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**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

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Purity Protein A purified

Clonality Monoclonal

Clone number EP197Y

Isotype IgG

Applications

The Abpromise guarantee Our

Our **Abpromise guarantee** covers the use of ab76299 in the following tested applications.

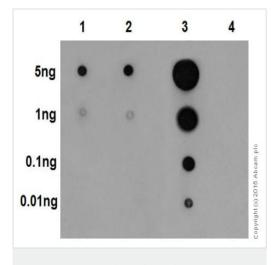
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/5000 - 1/10000. Detects a band of approximately 43, 42 kDa (predicted molecular weight: 43, 41 kDa).
IP		1/80.
Dot blot		Use at an assay dependent concentration.
ELISA		Use at an assay dependent concentration.

Application notes

Is unsuitable for Flow Cyt,ICC/IF or IHC-P.

Images

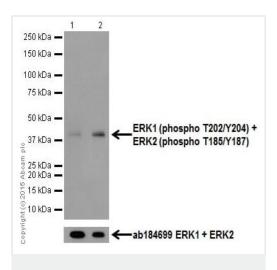


Dot Blot - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299)

Dot blot analysis of single phospho peptide pT202 (Lane 1), single phospho peptide pY204 (Lane 2), double phospho peptide pT202/pY204 (Lane 3) and non-phospho peptide (Lane 4) labelling Erk1 (pT202/pY204) + Erk2 (p185/pY187) with ab76299 at a dilution of 1/1000. A peroxidase-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody at a dilution of 1/1000.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Western blot - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299)

250 kDa =

150 kDa -100 kDa -

75 kDa 🕳

50 kDa -

20 kDa -15 kDa — 10 kDa =

은 37 kDa 🗕 25 kDa - **All lanes :** Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299) at 1/5000 dilution

Lane 1: A431 whole cell lysate - untreated

Lane 2: A431 whole cell lysate - treated with 100ng/ml EGF for 10

minutes

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000

dilution

Predicted band size: 43, 41 kDa Observed band size: 44 kDa

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.

All lanes: Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187)

antibody [EP197Y] (ab76299) at 1/10000 dilution

Lane 1: SH-SY5Y whole cell lysate - untreated

Lane 2: SH-SY5Y whole cell lysate - treated with 100ng/ml NGF for

10 minutes

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000

dilution

Western blot - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299)

■ ■ ← ab184699 ERK1 + ERK2

ERK1 (phospho T202/Y204) +

ERK2 (phospho T185/Y187)

Predicted band size: 43, 41 kDa Observed band size: 42,44 kDa

Exposure time: 15 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

All lanes : Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299) at 1/10000 dilution

Lane 1: PC-12 whole cell lysate - untreated

Lane 2: PC-12 whole cell lysate - treated with 100ng/ml NGF for 10 minutes

Lysates/proteins at 10 µg per lane.



All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000

dilution

Predicted band size: 43,41 kDa **Observed band size:** 42,44 kDa

Exposure time: 15 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

All lanes : Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299) at 1/10000 dilution

Lane 1: HeLa whole cell lysate - untreated

Lane 2: HeLa whole cell lysate - treated with Lambda

Phosphatase

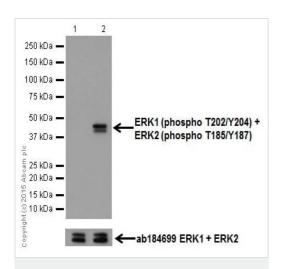
Lysates/proteins at 10 µg per lane.

Secondary

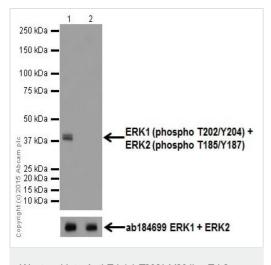
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000

dilution

Predicted band size: 43, 41 kDa Observed band size: 42,44 kDa



Western blot - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299)



Western blot - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299)

Exposure time: 30 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299) at 1/10000 dilution + NIH/3T3 whole cell lysate at 10 μ g

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 43, 41 kDa **Observed band size:** 42,44 kDa

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.

ab76299 at 1/80 immunoprecipitating Erk1 (pT202/pY204) + Erk2 (pT185/pY187) in A431 whole cell lysate.

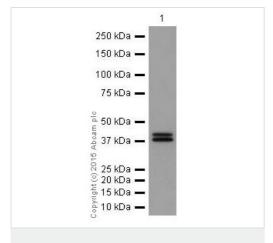
Lane 1 (input): A431 whole cell lysate - treated with 100ng/ml EGF for 10 min (10 μ g).

Lane 2 (+): ab76299 + A431 whole cell lysate - treated with 100ng/ml EGF for 10 min.

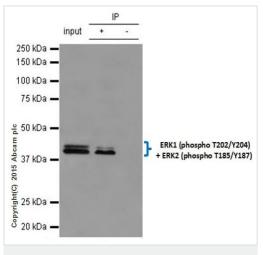
Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab76299 in A431 whole cell lysate - treated with 100ng/ml EGF for 10 min.

For western blotting, ab76299 was used at a dilution of 1/1000 and a HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of lgG, was used as the secondary antibody (1/1500).

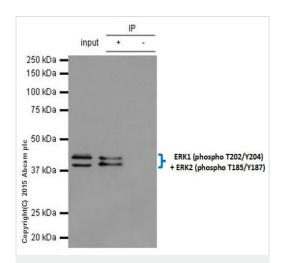
Blocking and dilution buffer: 5% NFDM/TBST.



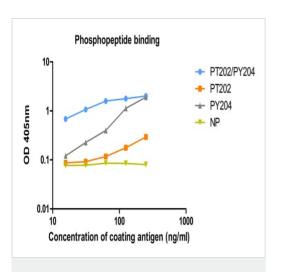
Western blot - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299)



Immunoprecipitation - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299)



Immunoprecipitation - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299)



ELISA - Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187) antibody [EP197Y] (ab76299)

ab76299 at 1/80 immunoprecipitating Erk1 (pT202/pY204) + Erk2 (pT185/pY187) in PC-12 whole cell lysate.

Lane 1 (input): PC-12 whole cell lysate - treated with 100ng/ml NGF for 10 min ($10\mu g$).

Lane 2 (+): ab76299 + PC-12 whole cell lysate - treated with 100ng/ml NGF for 10 min.

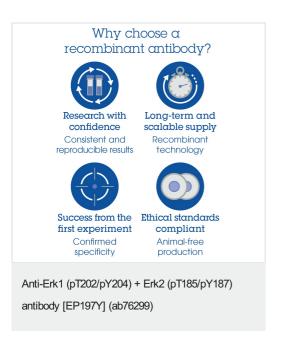
Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab76299 in PC-12 whole cell lysate - treated with 100ng/ml NGF for 10 min.

For western blotting, ab76299 was used at a dilution of 1/1000 and a HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of lgG, was used as the secondary antibody (1/1500).

Blocking and dilution buffer: 5% NFDM/TBST.

ELISA analysis of various phospho and non-phospho peptides (0-250 ng/ml) labelling Erk1 (pT202/pY204) + Erk2 (pT185/pY187) with ab76299 at a dilution of 1/1000. An Alkaline Phosphatase-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/2500).

ab76299 has stronger affinity for the double phospho peptide Y204/Y187 than to single phospho peptides T202 or Y204.



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