abcam

Product datasheet

Anti-EEF2/Elongation factor 2 antibody [EP880Y] ab75748

Recombinant RabMAb

6 References 11 Images

Overview

Product name Anti-EEF2/Elongation factor 2 antibody [EP880Y]

Description Rabbit monoclonal [EP880Y] to EEF2/Elongation factor 2

Host species Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide within Human EEF2/Elongation factor 2 (C terminal). The exact sequence is

proprietary.

Positive control A431, NIH3T3, HeLa, C6, PC-12 and NIH/3T3 cell lysates. Kidney carcinoma tissue, rat

pancreas, mouse pancreas, human glioma.

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[®] 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), 0.1% BSA

Purity Protein A purified

Clonality Monoclonal

1

Clone number EP880Y

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab75748 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)		1/70. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		1/10000 - 1/50000. Detects a band of approximately 95 kDa (predicted molecular weight: 95 kDa).
IP		1/20.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/100 - 1/250.

Target

Function Catalyzes the GTP-dependent ribosomal translocation step during translation elongation. During

this step, the ribosome changes from the pre-translocational (PRE) to the post-translocational (POST) state as the newly formed A-site-bound peptidyl-tRNA and P-site-bound deacylated tRNA move to the P and E sites, respectively. Catalyzes the coordinated movement of the two tRNA

molecules, the mRNA and conformational changes in the ribosome.

Sequence similaritiesBelongs to the GTP-binding elongation factor family. EF-G/EF-2 subfamily.

Post-translational Phosphorylation by EF-2 kinase completely inactivates EF-2.

modifications Diphthamide is 2-[3-carboxyamido-3-(trimethyl-ammonio)propyl]histidine. Diphthamide can be

ADP-ribosylated by diphtheria toxin and by Pseudomonas exotoxin A, thus arresting protein

synthesis.

ISGylated.

Cellular localization Cytoplasm.

Images



Western blot - Anti-EEF2/Elongation factor 2 antibody [EP880Y] (ab75748)

All lanes : Anti-EEF2/Elongation factor 2 antibody [EP880Y] (ab75748) at 1/50000 dilution

Lane 1: C6 (rat glioma) whole cell lysates

Lane 2: PC-12 (rat adrenal gland pheochromocytoma) whole cell

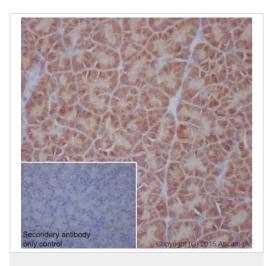
Lane 3: NIH/3T3 (mouse embryo) whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Rabbit monoclonal [EP880Y] to EEF2/Elongation factor 2 (ab75748) at 1/100000 dilution

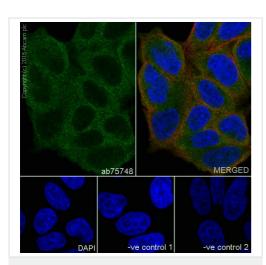
Predicted band size: 95 kDa



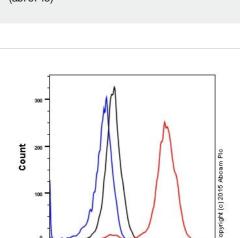
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EEF2/Elongation factor 2 antibody [EP880Y] (ab75748)

ab75748 staining EEF2/Elongation factor 2 in rat pancreas tissue sections by Immunohistochemistry (IHC-P - paraformaldehydefixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/700. A goat anti-rabbit IgG H&L (HRP) ab97051 was used as the secondary antibody at a dilution of 1/500.

Negative control 1: PBS in place of primary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-EEF2/Elongation factor 2 antibody [EP880Y] (ab75748)



Flow Cytometry (Intracellular) - Anti-EEF2/Elongation factor 2 antibody [EP880Y] (ab75748)

EEF2- Alexa Fluor® 488 (525/30 BP)

ab75748 staining EEF2/Elongation factor 2 in HeLa (human cervix adenocarcinoma) cells by ICC/IF

(Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti rabbit lgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody. ab7291 and ab150120 were used as counterstains for primary antibody ab75748 and secondary antibody ab150077 respectively and DAPI was used as a nuclear counterstain.

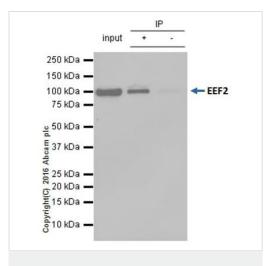
Negative control 1: Rabbit primary antibody and anti-mouse secondary antibody (<u>ab150120</u>)

Negative control 2: Mouse primary antibody (<u>ab7291</u>) and antirabbit secondary antibody (<u>ab150077</u>)

ab75748 stainingEEF2/Elongation factor 2 in the human cell line HEK293 (human embryonic kidney) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/70. A goat anti rabbit lgG (Alexa Fluor[®] 488) at a dilution of 1/500 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Immunoprecipitation - Anti-EEF2/Elongation factor 2 antibody [EP880Y] (ab75748)

ab75748 immunoprecipitating EEF2/Elongation factor 2. $10\mu g$ of cell lysate was incubated with primary antibody at a dilution of 1/20 and VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at a dilution of 1/10000.

Lane 1: HeLa (human cervix adenocarcinoma) whole cell lysate (10ug)

Lane 2: HeLa (human cervix adenocarcinoma) whole cell lysate

Lane 3: Rabbit monoclonal lgG (ab172730) instead of ab75748 in

HeLa (human cervix adenocarcinoma) whole cell lysate



Western blot - Anti-EEF2/Elongation factor 2 antibody [EP880Y] (ab75748)

All lanes : Anti-EEF2/Elongation factor 2 antibody [EP880Y] (ab75748) at 1/50000 dilution

Lane 1 : HEK293 (human embryonic kidney) whole cell lysates

Lane 2: HeLa (human cervix adenocarcinoma) whole cell lysates

Lane 3: A431 (human epidermoid carcinoma) whole cell lysates

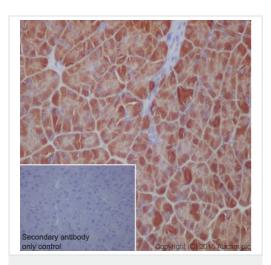
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 95 kDa

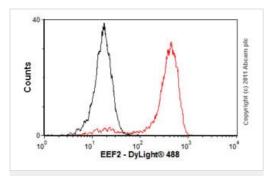
Additional bands at: 95 kDa. We are unsure as to the identity of these extra bands.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EEF2/Elongation factor 2 antibody [EP880Y] (ab75748)

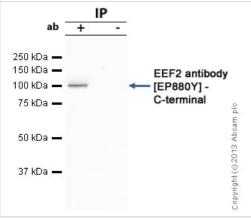
ab75748 staining EEF2/Elongation factor 2 in mouse pancreas tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/250. A goat anti-rabbit IgG H&L (HRP) ab97051 was used as the secondary antibody.

Negative control 1: PBS in place of primary antibody.



Flow Cytometry (Intracellular) - Anti-EEF2/Elongation factor 2 antibody [EP880Y] (ab75748)

Overlay histogram showing HeLa cells stained with ab75748 (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 (ab75748, 1µg/1x10 6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x10 6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Immunoprecipitation - Anti-EEF2/Elongation factor 2 antibody [EP880Y] (ab75748)

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EEF2/Elongation factor 2 antibody [EP880Y] (ab75748)

EEF2/Elongation factor 2 was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit monoclonal to EEF2/Elongation factor 2 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

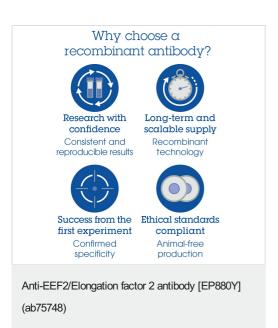
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab75748.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).

Band: 95kDa; EEF2/Elongation factor 2

ab75748 staining EEF2/Elongation factor 2 in human glioma tissue sections by Immunohistochemistry (IHC-P - paraformaldehydefixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/250. A goat anti-rabbit IgG H&L (HRP) ab97051 was used as the secondary antibody.

Negative control 1: PBS in place of primary antibody.



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