abcam

Product datasheet

Anti-MEF2D antibody [EPR24993-10] ab282731

KO VALIDATED Recombinant RabMAb

13 Images

Overview

Product name	Anti-MEF2D antibody [EPR24993-10]
Description	Rabbit monoclonal [EPR24993-10] to MEF2D
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IHC-P **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: THP-1, RAW 264.7, 2.4G2, His-tagged human MEF2D recombinant protein lysates. IHC-P: Mouse testis, Mouse cerebrum, Rat colon, and Rat cerebrum tissues. ICC/IF: THP-1, RAW 264.7 cells. Flow Cyt(Intra): THP-1, RAW 264.7 cells.
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: 7.2 Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR24993-10

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab282731 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)		1/500.
ICC/IF		1/50.
WB		1/1000. Predicted molecular weight: 56 kDa.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes Is unsuitable for IP.

Target

Function Transcriptional activator which binds specifically to the MEF2 element, 5'-YTA[AT](4)TAR-3', found in numerous muscle-specific, growth factor- and stress-induced genes. Mediates cellular functions not only in skeletal and cardiac muscle development, but also in neuronal differentiation and survival. Plays diverse roles in the control of cell growth, survival and apoptosis via p38 MAPK signaling in muscle-specific and/or growth factor-related transcription. Plays a critical role in the regulation of neuronal apoptosis.

Sequence similarities Belongs to the MEF2 family.
Contains 1 MADS-box domain.
Contains 1 Mef2-type DNA-binding domain.

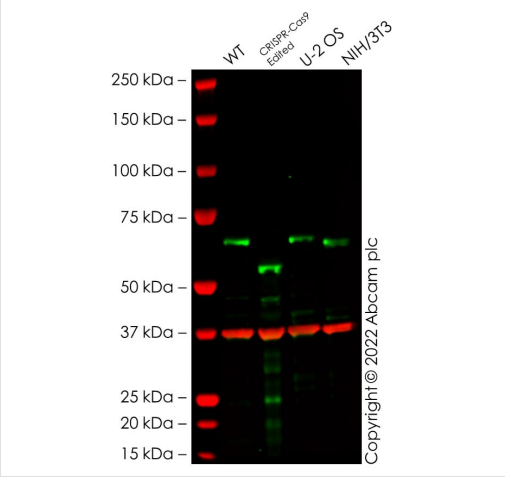
Developmental stage Present in myotubes and also in undifferentiated myoblasts.

Domain The beta domain, missing in a number of isoforms, is required for enhancement of transcriptional activity.

Post-translational modifications Phosphorylated on Ser-444 by CDK5 is required for Lys-439 sumoylation and inhibits transcriptional activity. In neurons, enhanced CDK5 activity induced by neurotoxins promotes caspase 3-mediated cleavage leading to neuron apoptosis. Phsophorylation on Ser-180 can be enhanced by EGF.
Acetylated on Lys-439 by CREBBP. Deacetylated by SIRT1.
Sumoylated on Lys-439 by SUMO2 but not SUMO1; which inhibits transcriptional activity and myogenic activity. Desumoylated by SENP3.
Proteolytically cleaved in cerebellar granule neurons on several sites by caspase 7 following neurotoxicity. Preferentially cleaves the CDK5-mediated hyperphosphorylated form which leads to neuron apoptosis and transcriptional inactivation.

Cellular localization Nucleus. Translocated by HDAC4 to nuclear dots.

Images



Western blot - Anti-MEF2D antibody [EPR24993-10] (ab282731)

All lanes : Anti-MEF2D antibody [EPR24993-10] (ab282731) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : mef2d CRISPR-Cas9 edited HeLa cell lysate
Lane 3 : U-2 OS cell lysate
Lane 4 : NIH/3T3 cell lysate

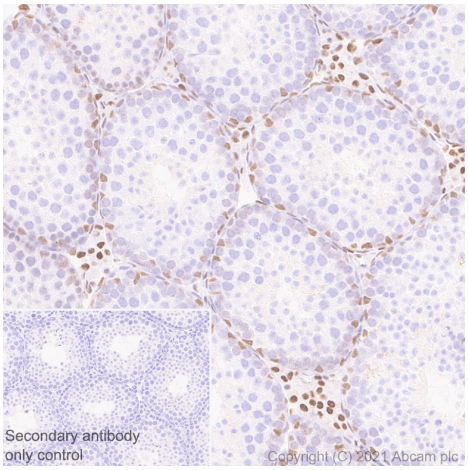
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 56 kDa
Observed band size: 67 kDa

False colour image of Western blot: Anti-MEF2D antibody [EPR24993-10] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab282731 was shown to bind specifically to MEF2D. A band was observed at 67 kDa in wild-type HeLa cell lysates with no signal observed at this size in mef2d CRISPR-Cas9 edited cell line **ab281636**. The band observed in the CRISPR-Cas9 edited lysate lane below 67 kDa is likely to represent a truncated form of MEF2D. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and mef2d CRISPR-Cas9 edited HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

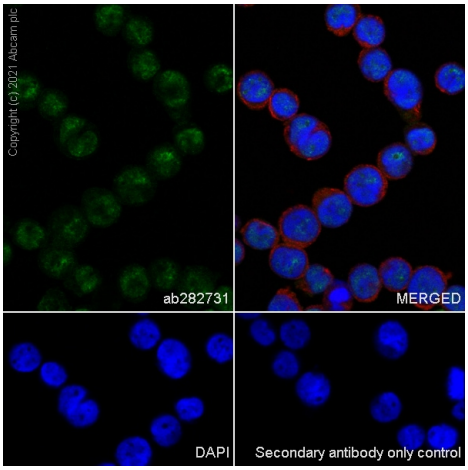


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEF2D antibody [EPR24993-10] (ab282731)

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labelling MEF2D with ab282731 at 1/2000 (0.286 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on mouse testis (PMID: 24694307). The section was incubated with ab282731 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

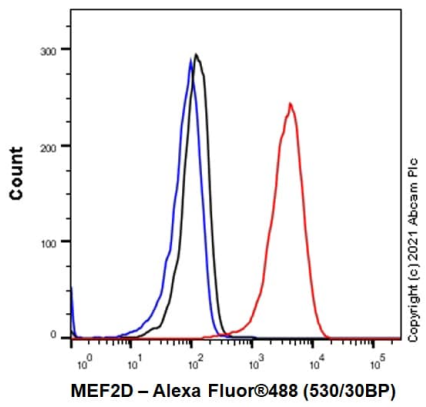


Immunocytochemistry/ Immunofluorescence - Anti-MEF2D antibody [EPR24993-10] (ab282731)

Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized THP-1 cells labelling MEF2D with ab282731 at 1/50 (11.42 ug/ml) dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green) Confocal image showing mainly nuclear staining in THP-1 cells is observed.

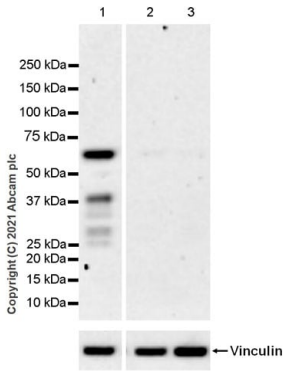
ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-MEF2D antibody [EPR24993-10] (ab282731)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized THP-1 (Human monocytic leukemia monocyte) cells labelling MEF2D with ab282731 at 1/500 dilution (0.1 ug) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-MEF2D antibody [EPR24993-10] (ab282731)

All lanes : Anti-MEF2D antibody [EPR24993-10] (ab282731) at 1/1000 dilution

Lane 1 : THP-1 (Human monocytic leukemia monocyte) whole cell lysates
Lane 2 : SW480 (human colorectal adenocarcinoma epithelial cell) whole cell lysate
Lane 3 : DLD-1 (human colon epithelial) whole cell lysate

Lysates/proteins at 20 µg per lane.

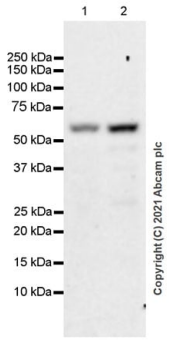
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 56 kDa
Observed band size: 70, 40 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Low expression: SW480/ DLD-1 (PMID: 27364559).

Exposure time: 147 seconds



Western blot - Anti-MEF2D antibody [EPR24993-10] (ab282731)

All lanes : Anti-MEF2D antibody [EPR24993-10] (ab282731) at 1/1000 dilution

Lane 1 : RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 2 : 2.4G2 (rat B cell lymphoma B lymphocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

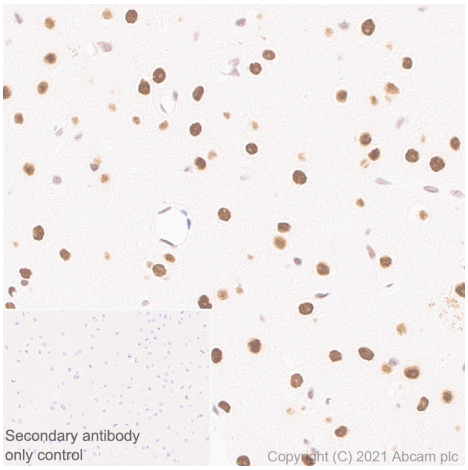
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 56 kDa
Observed band size: 70 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Lysates were made freshly and used in WB test immediately to minimize protein degradation.

Exposure time: 147 seconds

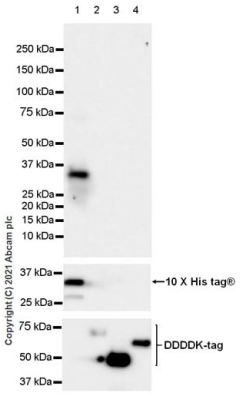


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEF2D antibody [EPR24993-10] (ab282731)

Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labelling MEF2D with ab282731 at 1/2000 (0.286 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on mouse cererbum (PMID: 28738418). The section was incubated with ab282731 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Western blot - Anti-MEF2D antibody [EPR24993-10] (ab282731)

All lanes : Anti-MEF2D antibody [EPR24993-10] (ab282731) at 1/1000 dilution

Lane 1 : His-tagged human MEF2D recombinant protein
Lane 2 : Myc/DDDDK-tagged human MEF2A recombinant protein
Lane 3 : Myc/DDDDK-tagged human MEF2B recombinant protein
Lane 4 : Myc/DDDDK-tagged human MEF2C recombinant protein

Lysates/proteins at 0.01 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 56 kDa
Observed band size: 31 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

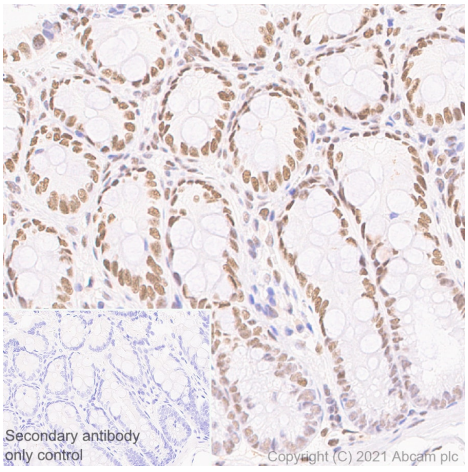
His-tagged human MEF2D recombinant protein (aa54-328) 10ng

Myc/DDDDK-tagged human MEF2A recombinant protein 10ng

Myc/DDDDK-tagged human MEF2B recombinant protein 10ng

Myc/DDDDK-tagged human MEF2C recombinant protein 10ng

Exposure time: 10 seconds

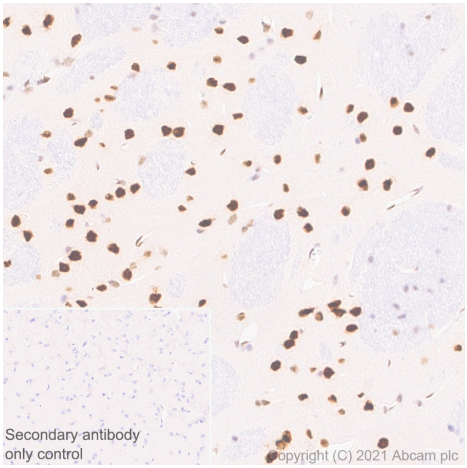


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEF2D antibody [EPR24993-10] (ab282731)

Immunohistochemical analysis of paraffin-embedded Rat colon tissue labelling MEF2D with ab282731 at 1/2000 (0.286 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on rat colon. The section was incubated with ab282731 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

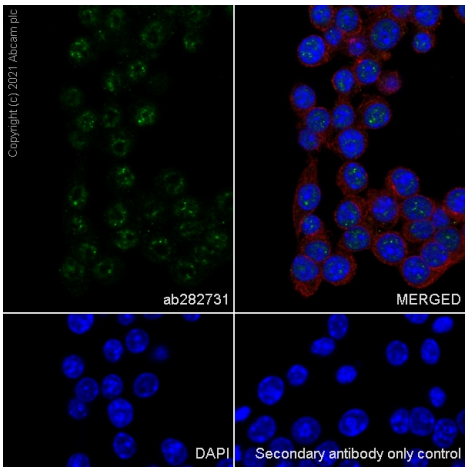


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEF2D antibody [EPR24993-10] (ab282731)

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labelling MEF2D with ab282731 at 1/2000 (0.286 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining on rat cererbum (PMID: 28738418). The section was incubated with ab282731 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

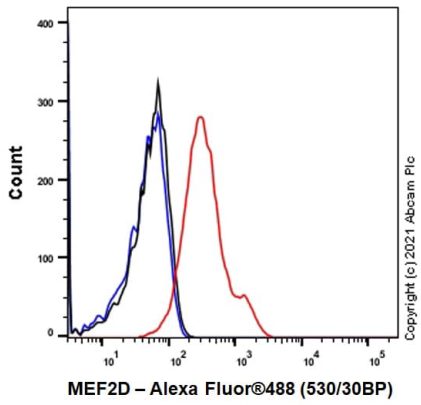


Immunocytochemistry/ Immunofluorescence - Anti-MEF2D antibody [EPR24993-10] (ab282731)

Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized RAW 264.7 cells labelling MEF2D with ab282731 at 1/50 (11.42 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green) Confocal image showing mainly nuclear staining in RAW 264.7 cells is observed.

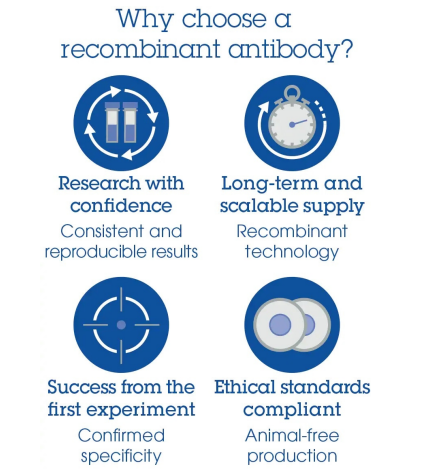
ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-MEF2D antibody [EPR24993-10] (ab282731)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells labelling MEF2D with ab282731 at 1/500 dilution (0.1 ug) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Anti-MEF2D antibody [EPR24993-10] (ab282731)

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors