abcam

Product datasheet

Anti-FMRP antibody [EPR23852-90] ab259335

KO VALIDATED Recombinant RabMAb

2 References 23 Images

Overview

Product name	Anti-FMRP antibody [EPR23852-90]
Description	Rabbit monoclonal [EPR23852-90] to FMRP
Host species	Rabbit
Tested applications	**Suitable for:** IP, Flow Cyt (Intra), IHC-Fr, IHC-P, WB, mIHC, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HeLa, NIH/3T3 and PC-12 whole cell lysates; Mouse brain and hippocampus tissue lysates; Rat hippocampus tissue lysate. IHC-P: Mouse cerebrum, and testis tissue; rat cerebrum and testis tissue. IHc (Frozen sections) - Mouse and Rat brain and testis. ICC/IF: C2C12 cells. Flow Cyt (intra): C2C12 cells. IP: HeLa and C2C12 whole cell lysates. mIHC: Mouse and rat cerebrum, Mouse and rat cerebellum tissue.
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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23852-90

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab259335 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
IP		1/30.
Flow Cyt (Intra)		Use at an assay dependent concentration.
IHC-Fr		Use a concentration of 0.5 µg/ml.
IHC-P		1/10000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 75, 80 kDa (predicted molecular weight: 69 kDa).
mIHC		Use at an assay dependent concentration.
ICC/IF		1/50.

Target

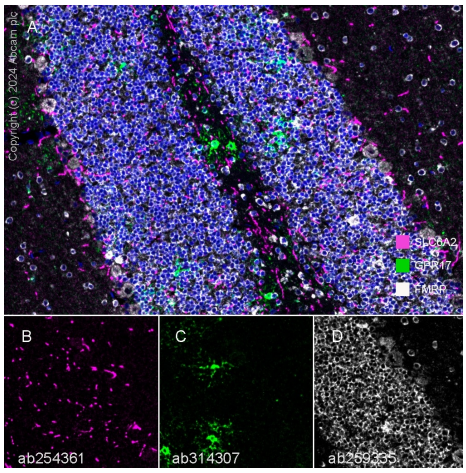
Function Translation repressor. Component of the CYFIP1-EIF4E-FMR1 complex which binds to the mRNA cap and mediates translational repression. In the CYFIP1-EIF4E-FMR1 complex this subunit mediates translation repression (By similarity). RNA-binding protein that plays a role in intracellular RNA transport and in the regulation of translation of target mRNAs. Associated with polysomes. May play a role in the transport of mRNA from the nucleus to the cytoplasm. Binds strongly to poly(G), binds moderately to poly(U) but shows very little binding to poly(A) or poly(C).

Tissue specificity Highest levels found in neurons, brain, testis, placenta and lymphocytes. Also expressed in epithelial tissues and at very low levels in glial cells.

Involvement in disease Defects in FMR1 are the cause of fragile X syndrome (FRAX) [MIM:300624]. Fragile X syndrome is a common genetic disease (has a prevalence of one in every 2000 children) which is characterized by moderate to severe mental retardation, macroorchidism (enlargement of the testicles), large ears, prominent jaw, and high-pitched, jocular speech. The defect in most fragile X syndrome patients results from an amplification of a CGG repeat region which is directly in front of the coding region.
Defects in FMR1 are the cause of fragile X tremor/ataxia syndrome (FXTAS) [MIM:300623]. In FXTAS, the expanded repeats range in size from 55 to 200 repeats and are referred to as 'premutations'. Full repeat expansions with greater than 200 repeats results in fragile X mental retardation syndrome [MIM:300624]. Carriers of the premutation typically do not show the full fragile X syndrome phenotype, but comprise a subgroup that may have some physical features of fragile X syndrome or mild cognitive and emotional problems.
Defects in FMR1 are the cause of premature ovarian failure syndrome type 1 (POF1) [MIM:311360]. An ovarian disorder defined as the cessation of ovarian function under the age of

	40 years. It is characterized by oligomenorrhea or amenorrhea, in the presence of elevated levels of serum gonadotropins and low estradiol.
Sequence similarities	Belongs to the FMR1 family. Contains 2 KH domains.
Post-translational modifications	Phosphorylated on several serine residues.
Cellular localization	Cytoplasm. Nucleus > nucleolus.

Images

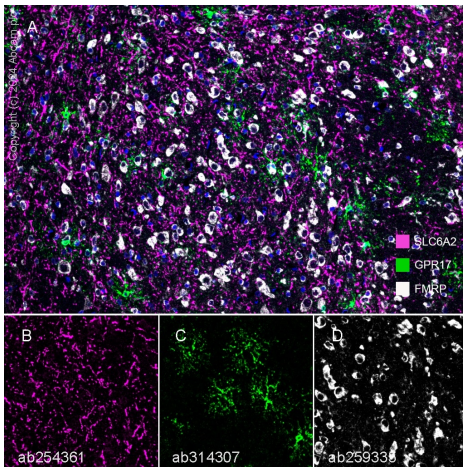


Multiplex immunohistochemistry - Anti-FMRP antibody [EPR23852-90] (ab259335)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat cerebellum labelling Noradrenaline transporter with **ab254361** at 1/100 (B), GPR17 with **ab314307** at 1/2000 dilution (C) and FMRP with ab259335 at 1/10000 dilution (D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and Nuclear DNA was labeled with DAPI (shown in blue). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-Noradrenaline transporter (magenta; Opal™690), anti-GPR17 (green; Opal™520) and anti-FMRP (gray; Opal™570) on rat cerebellum.
Panel B: anti-Noradrenaline transporter staining nerves in rat cerebellum.
Panel C: anti-GPR17 staining oligodendrocytes in rat cerebellum.
Panel D: anti-FMRP staining neurons in rat cerebellum.

The section was incubated in three rounds of staining: in the order of **ab254361**, **ab314307** and ab259335 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



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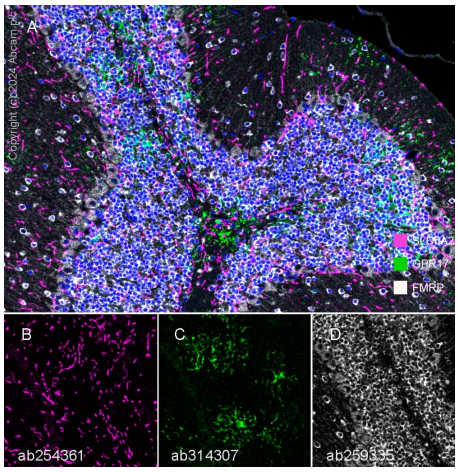

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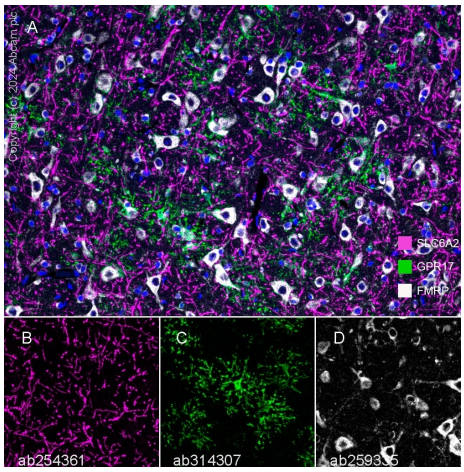

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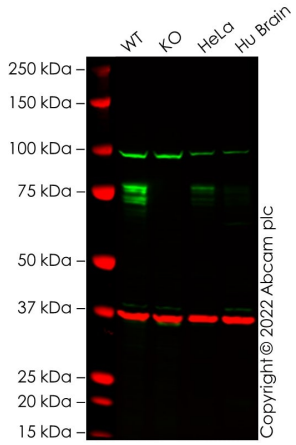


Western blot - Anti-FMRP antibody [EPR23852-90] (ab259335)

All lanes : Anti-FMRP antibody [EPR23852-90] (ab259335) at 1/1000 dilution

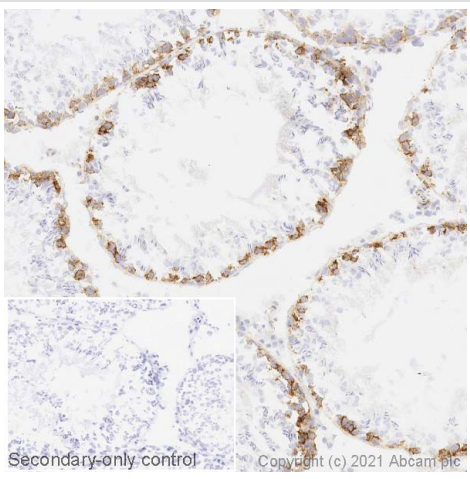
Lane 1 : Wild-type A549 cell lysate
Lane 2 : Fmr1 knockout A549 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Human Brain cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 69 kDa
Observed band size: 70-77 kDa

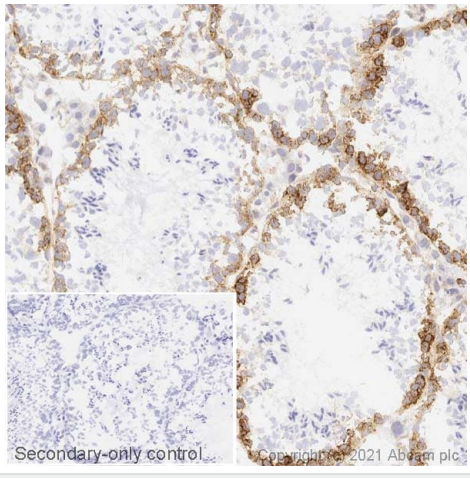
False colour image of Western blot: Anti-FMRP antibody [EPR23852-90] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab259335 was shown to bind specifically to FMRP. A band was observed at 70-77 kDa in wild-type A549 cell lysates with no signal observed at this size in Fmr1 knockout cell line **ab288956**. To generate this image, wild-type and Fmr1 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % 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 (Frozen sections) - Anti-FMRP antibody [EPR23852-90] (ab259335)

IHC image of ab259335 staining FMRP in rat testis frozen tissue sections, performed on a Leica Biosystems BOND® RX instrument. The section was incubated with ab259335 at 0.5µg/ml for 30 mins at room temperature and detected using an HRP conjugated compact polymer system (Bond™ Polymer Refine Detection). DAB was used as the chromogen. Positive staining was seen in cytoplasm and membrane in rat testis. 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.



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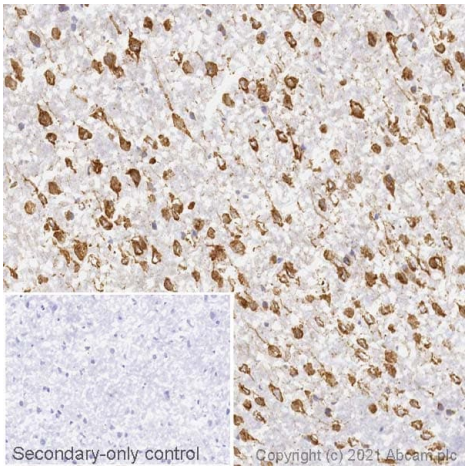

Immunohistochemistry (Frozen sections) - Anti-FMRP antibody [EPR23852-90] (ab259335)

IHC image of ab259335 staining FMRP in rat brain frozen tissue sections, performed on a Leica Biosystems BOND® RX instrument. The section was incubated with ab259335 at 0.5µg/ml for 30 mins at room temperature and detected using an HRP conjugated compact polymer system (Bond™ Polymer Refine Detection). DAB was used as the chromogen. Positive staining was seen in cytoplasm and membrane in rat brain. 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).

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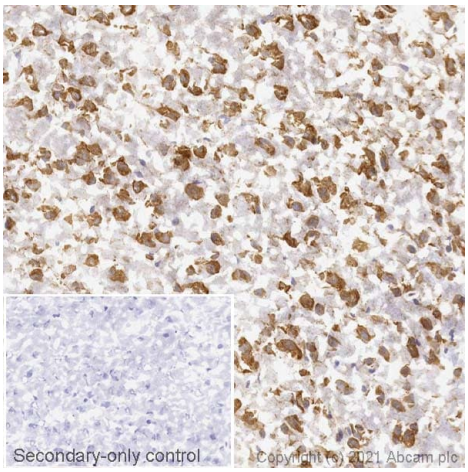

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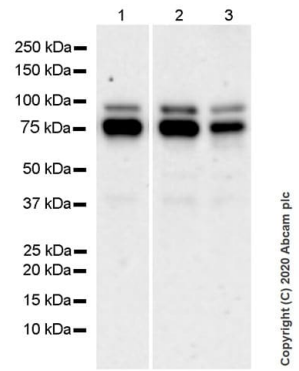


Western blot - Anti-FMRP antibody [EPR23852-90] (ab259335)

All lanes : Anti-FMRP antibody [EPR23852-90] (ab259335) at 1/1000 dilution

Lane 1 : Mouse brain tissue lysate
Lane 2 : Mouse hippocampus tissue lysate
Lane 3 : Rat hippocampus tissue lysate

Lysates/proteins at 20 µg per lane.

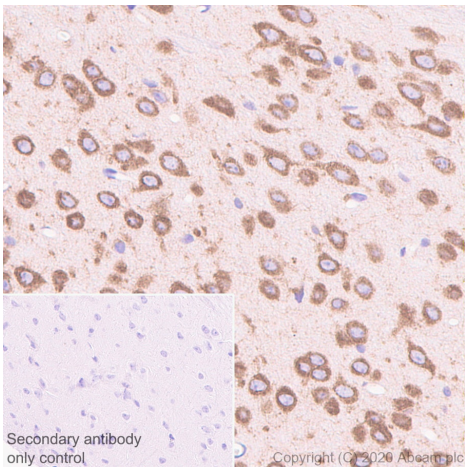
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 69 kDa
Observed band size: 75,80 kDa

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

Multiple bands could be seen due to alternative splicing of FMRP.

Exposure time: 180 seconds.

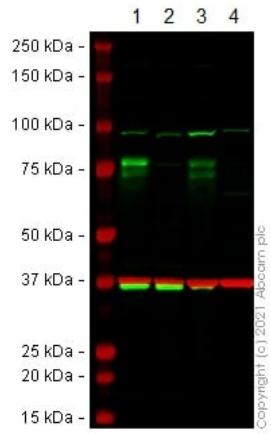


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FMRP antibody [EPR23852-90] (ab259335)

Immunohistochemical analysis of paraffin-embedded mouse cerebrum tissue labeling FMRP with ab259335 at 1/10000 (0.04 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on mouse cerebrum (PMID: 24463622). The section was incubated with ab259335 for 10 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 Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



Western blot - Anti-FMRP antibody [EPR23852-90] (ab259335)

All lanes : Anti-FMRP antibody [EPR23852-90] (ab259335) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : FMRP CRISPR/Cas9 edited HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Human brain cell lysate

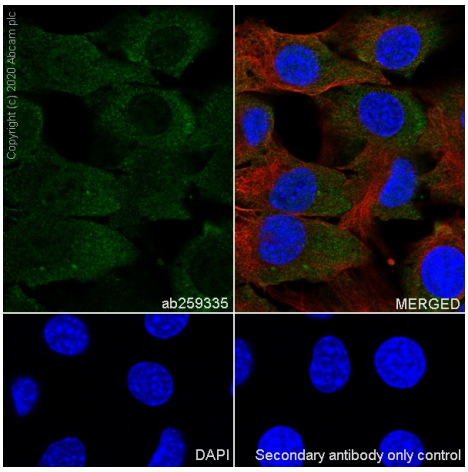
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 69 kDa
Observed band size: 77 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab259335 observed at 77 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

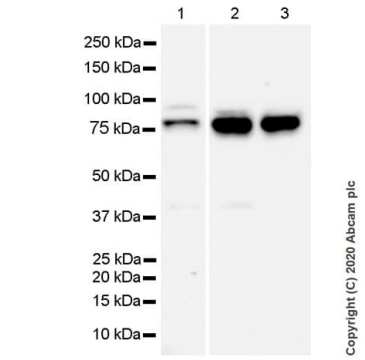
ab259335 was shown to react with FMRP in western blot. The band observed in the CRISPR/Cas9 edited lysate lane below 77 kDa is likely to represent a truncated form. This has not been investigated further. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab259335 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-FMRP antibody [EPR23852-90] (ab259335)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized C2C12 cells labelling FMRP with ab259335 at 1/50 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing strong cytoplasmic and weak nuclear staining in C2C12 cells. **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.



Western blot - Anti-FMRP antibody [EPR23852-90] (ab259335)

All lanes : Anti-FMRP antibody [EPR23852-90] (ab259335) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : NIH/3T3 (mouse embryonic fibroblast), whole cell lysate
Lane 3 : PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate

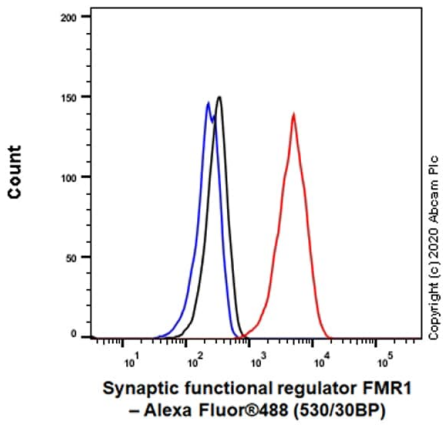
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 69 kDa
Observed band size: 80 kDa

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

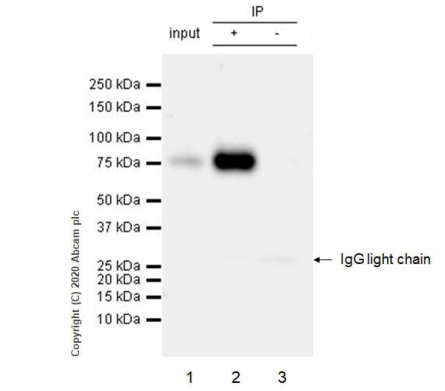
Exposure time: 26 seconds.



Flow Cytometry (Intracellular) - Anti-FMRP antibody [EPR23852-90] (ab259335)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized C2C12 (Mouse myoblast) cells labelling FMRP with ab259335 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**) isotype control (Black) 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.



Immunoprecipitation - Anti-FMRP antibody [EPR23852-90] (ab259335)

FMRP was immunoprecipitated from 0.35 mg C2C12 (Mouse myoblast) whole cell lysate 10ug with ab259335 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab259335 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

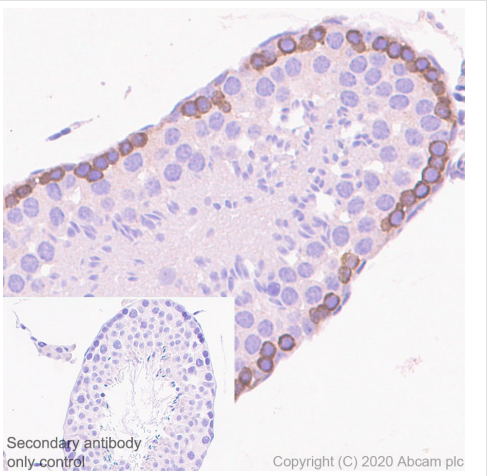
Lane 1: C2C12 (Mouse myoblast) whole cell lysate 10ug

Lane 2: ab259335 IP in C2C12 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab259335 in C2C12 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.

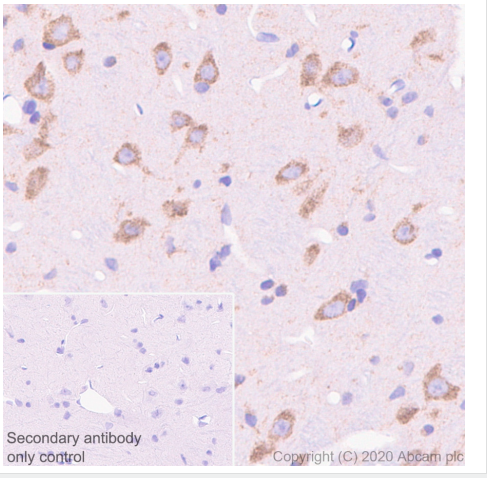


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FMRP antibody [EPR23852-90] (ab259335)

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling FMRP with ab259335 at 1/10000 (0.04 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on mouse testis (PMID: 16790844). The section was incubated with ab259335 for 10 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 Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

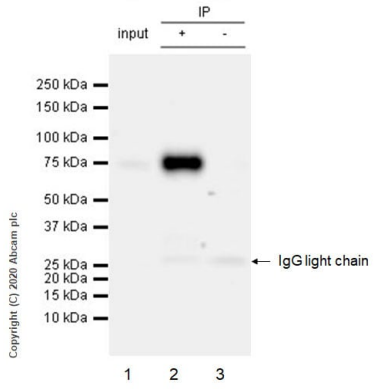


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FMRP antibody [EPR23852-90] (ab259335)

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling FMRP with ab259335 at 1/10000 (0.04 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on rat cerebrum. The section was incubated with ab259335 for 10 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 Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



Immunoprecipitation - Anti-FMRP antibody [EPR23852-90] (ab259335)

FMRP was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate with ab259335 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab259335 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

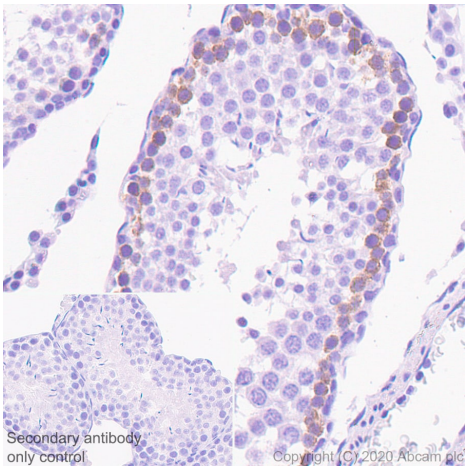
Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10ug

Lane 2: ab259335 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab259335 in HeLa whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.

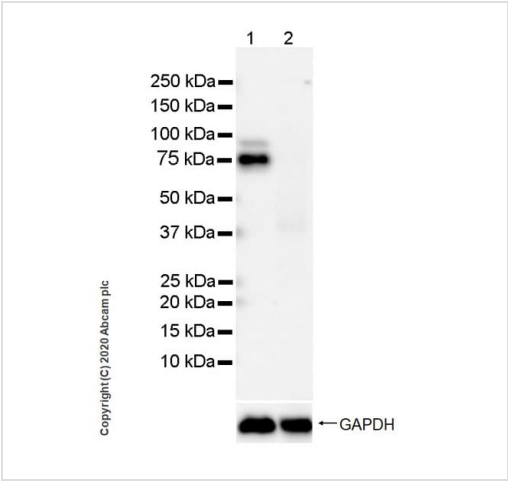


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FMRP antibody [EPR23852-90] (ab259335)

Immunohistochemical analysis of paraffin-embedded Rat testis tissue labeling FMRP with ab259335 at 1/10000 (0.04 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on rat testis. The section was incubated with ab259335 for 10 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 Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



Western blot - Anti-FMRP antibody [EPR23852-90] (ab259335)

All lanes : Anti-FMRP antibody [EPR23852-90] (ab259335) at 1/1000 dilution

Lane 1 : Mouse brain tissue lysate

Lane 2 : Mouse heart tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 69 kDa

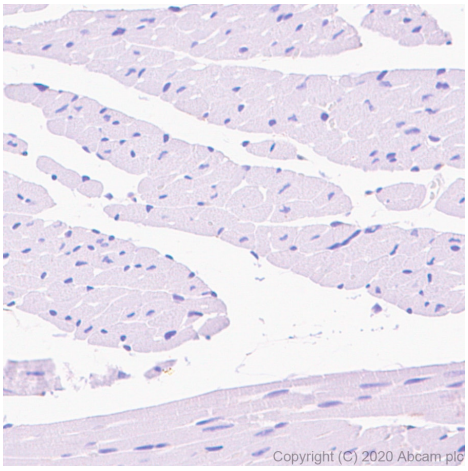
Observed band size: 75,80 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 81 seconds.

Negative control: Mouse heart(PMID: 7633436).

Multiple bands could be seen due to alternative splicing of FMRP

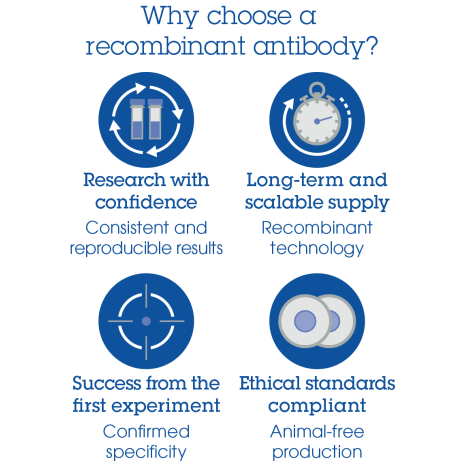


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FMRP antibody [EPR23852-90] (ab259335)

Immunohistochemical analysis of paraffin-embedded mouse cardiac muscle tissue labeling FMRP with ab259335 at 1/10000 (0.04 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). **Negative control:** No staining on mouse cardiac muscle (PMID: 7633436). The section was incubated with ab259335 for 10 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 Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.



Anti-FMRP antibody [EPR23852-90] (ab259335)

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