abcam

Product datasheet

Anti-FMRP antibody ab17722



★★★★★ 8 Abreviews 73 References 画像数 15

製品の概要

製品名 Anti-FMRP antibody

製品の詳細 Rabbit polyclonal to FMRP

由来種 Rabbit

アプリケーション 適用あり: IHC-P, WB, ICC/IF, IHC-FoFr, IP

種交差性 交差種: Mouse, Rat, Human

交差が予測される動物種: Orangutan 🔷

免疫原 Synthetic peptide within Human FMRP aa 550 to the C-terminus (internal sequence) conjugated

to keyhole limpet haemocyanin. The exact sequence is proprietary.

(Peptide available as ab19074)

ポジティブ・コントロール IHC-P: Human cerebral cortex tissue sections.

特記事項 <u>ab27455</u> does not recognize endogenous FMRP (expected size 71 kDa) in human testis lysate,

which may be due to low expression levels of FMRP.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

バッファー pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

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agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

精製度 Immunogen affinity purified

ポリ/モノ ポリクローナル

アイソタイプ IgG

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab17722の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
IHC-P	**** <u>(2)</u>	Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB	**** (2)	Use a concentration of 1 µg/ml. Detects a band of approximately 80 kDa (predicted molecular weight: 71 kDa).
ICC/IF	★★★★ <u>(2)</u>	Use a concentration of 5 µg/ml.
IHC-FoFr	★★★ ☆☆ <u>(2)</u>	Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

ターゲット情報

機能

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).

組織特異性

関連疾患

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

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.

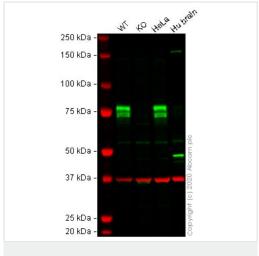
配列類似性 Belongs to the FMR1 family.

Contains 2 KH domains.

翻訳後修飾 Phosphorylated on several serine residues.

細胞内局在 Cytoplasm. Nucleus > nucleolus.

画像



Western blot - Anti-FMRP antibody (ab17722)

All lanes: Anti-FMRP antibody (ab17722) at 1 µg/ml

Lane 1: Wild-type HAP1 cell lysate

Lane 2: FMR1 knockout HAP1 cell lysate

Lane 3: HeLa cell lysate

Lane 4: Human Brain cell lysate

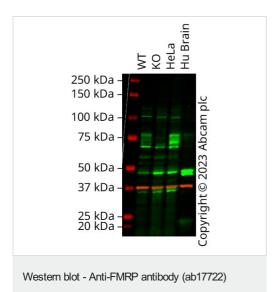
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 71 kDa Observed band size: 80 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab17722 observed at 80 kDa. Red - loading control, ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab17722 was shown to react with FMR1 in HAP1 wild-type cells in western blot. Loss of signal was observed when FMR1 knockout sample was used. HAP1 wild-type and FMR1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab17722 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 1 µg/ml 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 hour at room temperature before imaging.



All lanes: Anti-FMRP antibody (ab17722) at 1 µg

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: 71 kDa **Observed band size:** 77 kDa

False colour image of Western blot: Anti-FMRP antibody staining at 1 ug/ml, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab17722 was shown to bind specifically to FMRP. A band was observed at 77 kDa in wild-type A549 cell lysates with no signal observed at this size in Fmr1 knockout cell line. 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 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 lgG H&L 800CW and Goat anti-Mouse lgG H&L 680RD at 1/20000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-FMRP antibody (ab17722)

Secondary-only control Copyright (c) 2023 Abcam plc

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FMRP antibody (ab17722)

Lab

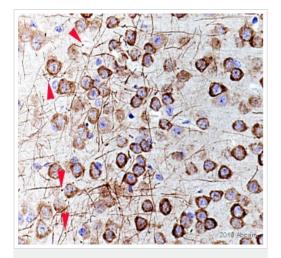
ab17722 stained in SK-N-SH (Human neuroblastoma cell line) cells.

Cells were fixed with 100% methanol (5 min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1h at room temperature to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab17722 at 5 µg/ml and ab7291 (Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150120 (pseudo-colored red) and ab150081 (colored green) used at 1 µg/ml for 1hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43µM for 1hour at room temperature.

IHC image of FMRP staining in a section of formalin-fixed paraffinembedded human normal cerebral cortex* performed on a Leica Biosystems BOND® RX instrument. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab17722, 5ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

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.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre."

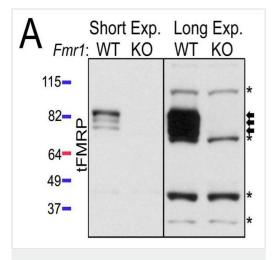


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FMRP antibody (ab17722)

This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom

IHC image of FMRP staining in mouse frontal cortex section.

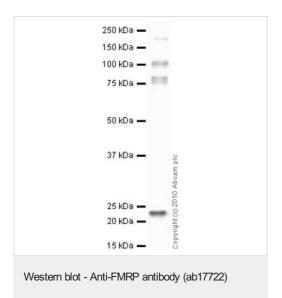
The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6). The section was then blocked using 1% BSA for 10 mins at 21°C The section was then incubated with ab17722, 1/1500, for 2 hours at 21°C. The section was then counterstained with hematoxylin.



Western blot - Anti-FMRP antibody (ab17722)

Bartley et al PLoS One. 2014 May 7;9(5):e96956. doi: 10.1371/journal.pone.0096956. eCollection 2014. Fig 1. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

The specificity of the C-terminal, phospho-insensitive FMRP tFMRP antibody (Abcam ab17722) was verified by immunoblotting whole cell cortical lysates from 2 month-old *Fmr1*^{y/+} (WT) and *Fmr1*^{y/-} (KO) mice. Short and long exposures (exp.) are shown. Arrows point to three FMRP-specific bands while asterisks point to nonspecific bands.



Anti-FMRP antibody (ab17722) at 1 μ g/ml + Brain (Mouse) Tissue Lysate at 10 μ g

Secondary

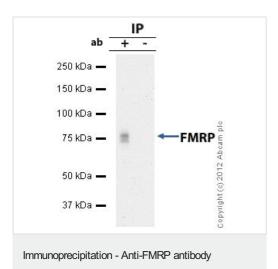
Goat polyclonal to Rabbit lgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 71 kDa **Observed band size:** 80 kDa

Additional bands at: 105 kDa, 23 kDa, 75 kDa (possible isoform). We are unsure as to the identity of these extra bands.



(ab17722)

Exposure time: 4 minutes

FMRP was immunoprecipitated using 0.5mg HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell extract, 5µg of Rabbit polyclonal to FMRP and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

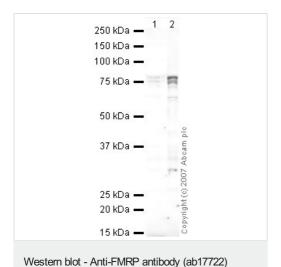
The antibody was incubated under agitation with Protein G beads for 10min, HeLa whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

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 ab17722.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to

Rabbit IgG light chain (HRP) (ab99697).

Band: 80kDa: FMRP.



All lanes: Anti-FMRP antibody (ab17722) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 2 : HeLa (Human epithelial carcinoma cell line) Nuclear

Lysate

Lysates/proteins at 10 µg per lane.

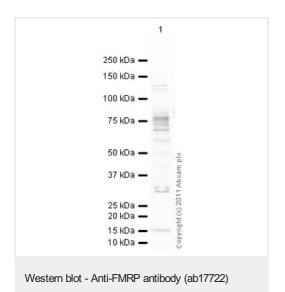
Secondary

All lanes : IRDye 680 Conjugated Goat Anti-Rabbit lgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 71 kDa **Observed band size:** 80 kDa

Additional bands at: 75 kDa (possible isoform)



Anti-FMRP antibody (ab17722) at 1 μ g/ml + PC-12 (Rat adrenal pheochromocytoma cell line) whole cell lysate at 20 μ g

Secondary

Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 71 kDa **Observed band size:** 80 kDa

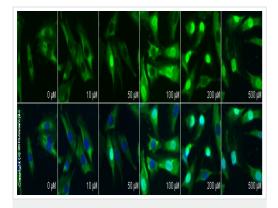
Additional bands at: 13 kDa, 32 kDa, 68 kDa. We are unsure as

to the identity of these extra bands.

FMRP Nudeolin DAPI Merge

Immunocytochemistry/ Immunofluorescence - Anti-FMRP antibody (ab17722)

Taha et al PLoS One. 2014 Mar 21;9(3):e91465. doi: 10.1371/journal.pone.0091465. eCollection 2014. Fig 1. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/



Immunocytochemistry/ Immunofluorescence - Anti-FMRP antibody (ab17722)

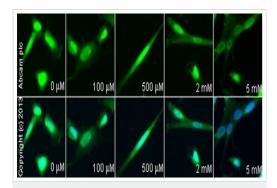
Exposure time: 2 minutes

FMRP is localized at various intracellular sites in HeLa cells.

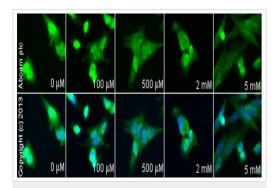
Confocal laser scanning microscopy (cLSM) images of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells depicting endogenous FMRP (ab17722, green channel) costained with various cytosolic (Panel A, not shown) and nuclear (Panel B, shown) markers (red channel). DNA was stained by using DAPI (blue channel). Boxed areas in the merged panels depict enlarged areas of interest. Scale bar: $10~\mu m$.

HeLa cells grown on glass coverslips were fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.25% triton X-100 in PBS for 10 min, and thereafter blocked for 1 h in a solution containing 3% BSA in 0.25% triton-X100/PBS. Cells were incubated with primary and secondary antibodies for 1 h and finally counterstained with DAPI for 5 min and mounted using the prolong gold anti-fade reagent.

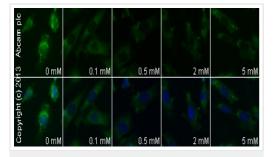
ab17722 staining FMRP in SK-N-SH (Human neuroblastoma cell line) cells treated with (R,S)-3,5-DHPG (ab120020), by ICC/IF. Increase in FMRP expression correlates with increased concentration of (R,S)-3,5-DHPG, as described in literature. The cells were incubated at 37°C for 1h in media containing different concentrations of ab120020 ((R,S)-3,5-DHPG) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab17722 (5 μ g/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Immunocytochemistry/ Immunofluorescence - Anti-FMRP antibody (ab17722)



Immunocytochemistry/ Immunofluorescence - Anti-FMRP antibody (ab17722)



Immunocytochemistry/ Immunofluorescence - Anti-FMRP antibody (ab17722)

ab17722 staining FMRP in SK-N-SH (Human neuroblastoma cell line) cells treated with (R,S)-MCPG sodium salt (**ab120252**), by ICC/IF. Decrease of FMRP expression correlates with increased concentration of (R,S)-MCPG sodium salt, as described in literature.

The cells were incubated at 37°C for 2h in media containing different concentrations of ab120252 ((R,S)-MCPG sodium salt) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab17722 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

ab17722 staining FMRP in SK-N-SH (Human neuroblastoma cell line) cells treated with (S)-MCPG (<u>ab120059</u>), by ICC/IF. Decrease of FMRP expression correlates with increased concentration of (S)-MCPG, as described in literature.

The cells were incubated at 37°C for 30 minutes in media containing different concentrations of **ab120059** ((S)-MCPG) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab17722 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (**ab96899**) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

ab17722 staining FMRP in SK-N-SH (Human neuroblastoma cell line) cells treated with (R,S)-MCPG ($\underline{ab120033}$), by ICC/IF. Decrease in FMRP expression correlates with increased concentration of (R,S)-MCPG, as described in literature. The cells were incubated at 37°C for 2h in media containing different concentrations of $\underline{ab120033}$ ((R,S)-MCPG) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab17722 (5 μ g/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody ($\underline{ab96899}$) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with

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