Product datasheet

Anti-FMRP antibody ab17722

Overview

Product name: Anti-FMRP antibody
Description: Rabbit polyclonal to FMRP
Host species: Rabbit
Tested applications: Suitable for: IHC-P, WB, IHC-FoFr, ICC/IF, IP
Species reactivity: Reacts with: Mouse, Rat, Human
Predicted to work with: Orangutan

Immunogen: Synthetic peptide conjugated to KLH derived from within residues 550 to the C-terminus of Human FMRP. Read Abcam's proprietary immunogen policy (Peptide available as ab19074.)

Positive control: This antibody gave a positive signal in the following lysates: HeLa Whole Cell, HeLa Nuclear, Mouse Brain Tissue, PC12 whole cell. ICC-IF: SKNSH and Hela cells.

General notes: ab27455 does not recognise endogenous FMRP (expected size 71 kDa) in human testes lysate, which may be due to low expression levels of FMRP.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer: Preservative: 0.02% Sodium Azide
Constituents: 1% BSA, PBS, pH 7.4
Purity: Immunogen affinity purified
Clonality: Polyclonal
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab17722 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
**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.

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<td>IHC-P</td>
<td></td>
<td>Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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<tr>
<td>WB</td>
<td></td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 80 kDa (predicted molecular weight: 71 kDa).</td>
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<tr>
<td>IHC-FoFr</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>ICC/IF</td>
<td></td>
<td>Use a concentration of 5 µg/ml.</td>
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<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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</table>
Anti-FMRP antibody (ab17722) at 1 µg/ml + Brain (Mouse) Tissue Lysate at 10 µg

**Secondary**
Goat polyclonal to Rabbit IgG - 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

*why is the actual band size different from the predicted?*

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

**Exposure time:** 4 minutes

ab17722 staining FMRP in SK-N-SH 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 µg/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.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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 haematoxylin.

Immunocytochemistry/ Immunofluorescence - Anti-FMRP antibody (ab17722)

ab17722 stained in SKNSH 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 permeabilise 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.
ab17722 staining FMRP in SK-N-SH 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 cells treated with (S)-MCPG (ab120059), 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 cells treated with (R,S)-MCPG (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 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 µg/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.
Western blot - Anti-FMRP antibody (ab17722)

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 IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

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

why is the actual band size different from the predicted?

Additional bands at: 75 kDa (possible isoform)

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

IHC image of FMRP staining in human tonsil FFPE section, performed on a Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab17722, 5µg/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.
Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-FMRP antibody (ab17722)

This image is courtesy of an Abreview submitted by Dr Sophie Pezet

ab17722 staining FMRP in rat brain tissue section by Immunohistochemistry (PFA perfusion fixed frozen sections). Tissue from 4% PFA perfused animals underwent overnight fixation in 4% paraformaldehyde, cryoprotected in 30% sucrose and cut using cryostat. The primary antibody was diluted, 1/300 (PBS + 0.3% Triton X100) and incubated with sample for 18 hours at 20°C. An abcam antibody ab60314, Chromeo 488 conjugated goat polyclonal to rabbit IgG, diluted 1/1000 was used as secondary.

Western blot - Anti-FMRP antibody (ab17722)

Anti-FMRP antibody (ab17722) at 1 µg/ml + PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate at 20 µg

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 why is the actual band size different from the predicted?
Additional bands at: 13 kDa, 32 kDa, 68 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 2 minutes
FMRP was immunoprecipitated using 0.5mg Hela 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.


Band: 80kDa: FMRP.

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