abcam

Product datasheet

Anti-M6PR (cation independent) antibody [2G11] ab2733

KO VALIDATED

18 Abreviews 123 References 6 Images

Overview

Product name	Anti-M6PR (cation independent) antibody [2G11]
Description	Mouse monoclonal [2G11] to M6PR (cation independent)
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra) **Unsuitable for:** WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Cow, Non human primates, African green monkey **Does not react with:** Hamster
Immunogen	Full length protein. This information is proprietary to Abcam and/or its suppliers.
Epitope	This antibody is shown to recognize an epitope in the extracellular domain of Mannose 6 Phosphate Receptor.
Positive control	Flow Cyt (Intra): A431 cells. ICC/IF: HAP1 cells (HAP1-IGF2R knockout cells used as negative cell line).
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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

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	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Clonality	Monoclonal
Clone number	2G11
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab2733 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

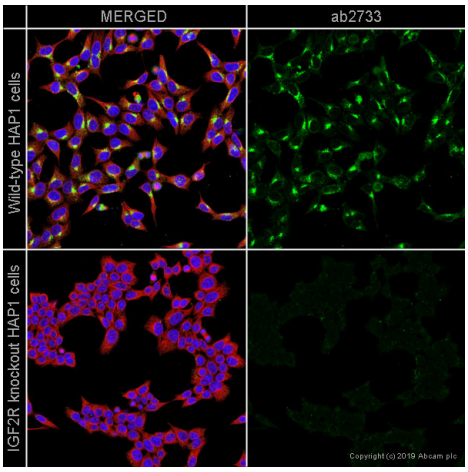
Application	Abreviews	Notes
ICC/IF	★★★★★ **(15)**	Use a concentration of 1 - 10 µg/ml.
Flow Cyt (Intra)		Use 1µg for 10^6 cells. **ab170191** - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

Application notes Is unsuitable for WB.

Target

Function	Transport of phosphorylated lysosomal enzymes from the Golgi complex and the cell surface to lysosomes. Lysosomal enzymes bearing phosphomannosyl residues bind specifically to mannose-6-phosphate receptors in the Golgi apparatus and the resulting receptor-ligand complex is transported to an acidic prelyosomal compartment where the low pH mediates the dissociation of the complex. This receptor also binds IGF2. Acts as a positive regulator of T-cell coactivation, by binding DPP4.
Sequence similarities	Belongs to the MRL1/IGF2R family. Contains 1 fibronectin type-II domain.
Domain	Contains 15 repeating units of approximately 147 AA harboring four disulfide bonds each. The most highly conserved region within the repeat consists of a stretch of 13 AA that contains cysteines at both ends.
Cellular localization	Lysosome membrane. Colocalized with DPP4 in internalized cytoplasmic vesicles adjacent to the cell surface.

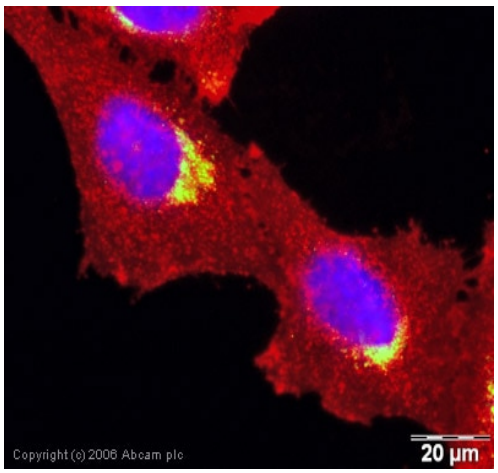
Images



Immunocytochemistry/ Immunofluorescence - Anti-M6PR (cation independent) antibody [2G11] (ab2733)

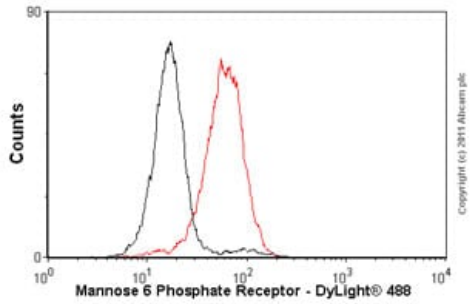
ab2733 staining IGF2R in wild-type HAP1 cells (top panel) and IGF2R knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab2733 at 1ug/ml and **ab6046** (Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 µg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (**ab150080**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



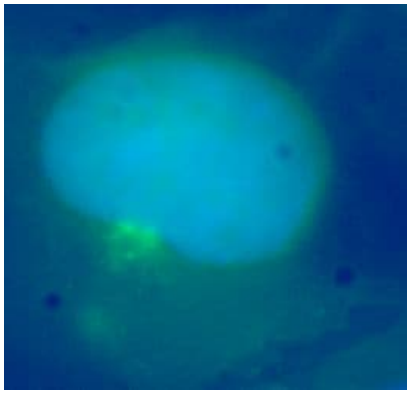
Immunocytochemistry/ Immunofluorescence - Anti-M6PR (cation independent) antibody [2G11] (ab2733)

ICC/IF image of ab2733 stained human HeLa cells. The cells were methanol fixed (5 min) and incubated with the antibody (ab2733, 1µg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT^{TM} FX Signal Enhancer was used as the primary blocking agent, 5% BSA (in TBS-T) was used for all other blocking steps. DAPI was used to stain the cell nuclei (blue). Alexa Fluor® 594 WGA was used to label plasma membranes (red).



Flow Cytometry (Intracellular) - Anti-M6PR (cation independent) antibody [2G11] (ab2733)

Overlay histogram showing HeLa cells stained with ab2733 (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 (ab2733, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (**ab91361**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

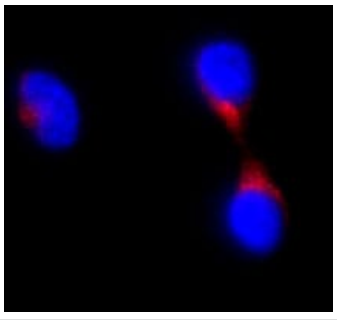


Immunocytochemistry/ Immunofluorescence - Anti-M6PR (cation independent) antibody [2G11] (ab2733)

Luke Hughes-Davies and Rhiannon Jade, Gurdon Institute, Cambridge, UK

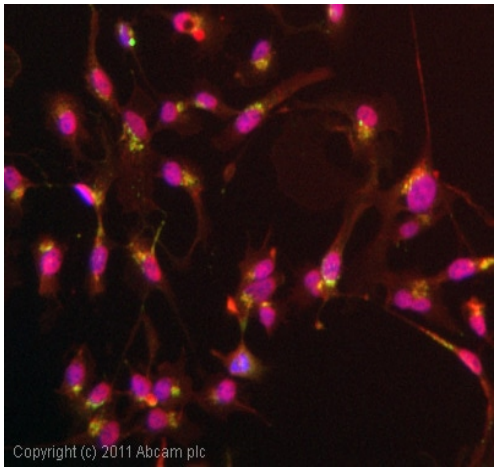
Immunofluorescent imaging of human cells (U2OS) with ab2733 confirms the specificity of this antibody, with the expected perinuclear vesicular staining of lysosomes.

IF was performed with a standard paraformaldehyde technique (fixed in PBS buffered PFH 4% for 5 minutes, permeabilised with 0.5% triton-PBS for 5 minutes, blocked with 5% milk / 0.2% tween for one hour. Primary antibody used at 1/100 in 5% milk / 0.2% TWEEN for one hour, secondary antibody for 30 minutes. All blocking and incubation steps carried out at 37 degrees.



Immunocytochemistry/ Immunofluorescence - Anti-M6PR (cation independent) antibody [2G11] (ab2733)

ab2733 positively staining formaldehyde fixed Human HEK 293 cells (red) in conjunction with goat anti mouse (Alexa 546). Nuclear staining was obtained using Hoechst.
This image is an edited version of an image received courtesy of an Abreview submitted by **Kun Liu on 19 September 2005**. We do not have any further information relating to this image.



Immunocytochemistry/ Immunofluorescence - Anti-M6PR (cation independent) antibody [2G11] (ab2733)

ICC/IF image of ab2733 stained HepG2 cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab2733, 10µg/ml) overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti-mouse IgG - H&L, pre-adsorbed (**ab96879**) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

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