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Product datasheet

Anti-Beta Arrestin 2 antibody ab54790

KO VALIDATED

★★★★★ 3 Abreviews 20 References 7 Images

Overview

Product name	Anti-Beta Arrestin 2 antibody
Description	Mouse monoclonal to Beta Arrestin 2
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt, IHC-P, WB, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment corresponding to Human Beta Arrestin 2 aa 300-409. Database link: P32121
Positive control	WB: HepG2, A549, K562 and HEK293T cell lysate and mouse brain tissue lysate. IHC-P: Human tonsil tissue. ICC/IF: HeLa cells. IP: HEK-293T cells.
General notes	This product was changed from ascites to tissue culture supernatant on 13th Feb 2019. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team. Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough. Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility. We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee. In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for. We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee. Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee. 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, as well as 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
Purity	Tissue culture supernatant
Clonality	Monoclonal
Isotype	IgG2a
Light chain type	kappa

Applications

Our Abpromise guarantee covers the use of **ab54790** 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		Use at an assay dependent concentration. ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration.
WB	★★★★★	Use at an assay dependent concentration. This antibody has only been tested in WB against the recombinant fragment used as immunogen. We have no data on the detection of endogenous protein.
ICC/IF	★★★★★	Use at an assay dependent concentration.

Target

Function

Functions in regulating agonist-mediated G-protein coupled receptor (GPCR) signaling by mediating both receptor desensitization and resensitization processes. During homologous desensitization, beta-arrestins bind to the GPRK-phosphorylated receptor and sterically preclude its coupling to the cognate G-protein; the binding appears to require additional receptor determinants exposed only in the active receptor conformation. The beta-arrestins target many receptors for internalization by acting as endocytic adapters (CLASPs, clathrin-associated sorting proteins) and recruiting the GPRCs to the adapter protein 2 complex 2 (AP-2) in clathrin-coated pits (CCPs). However, the extent of beta-arrestin involvement appears to vary significantly depending on the receptor, agonist and cell type. Internalized arrestin-receptor complexes traffic to intracellular endosomes, where they remain uncoupled from G-proteins. Two different modes of arrestin-mediated internalization occur. Class A receptors, like ADRB2, OPRM1, ENDRA, D1AR and ADRA1B dissociate from beta-arrestin at or near the plasma membrane and undergo rapid recycling. Class B receptors, like AVPR2, AGTR1, NTSR1, TRHR and TACR1 internalize as a complex with arrestin and traffic with it to endosomal vesicles, presumably as desensitized receptors, for extended periods of time. Receptor resensitization then requires that receptor-

bound arrestin is removed so that the receptor can be dephosphorylated and returned to the plasma membrane. Mediates endocytosis of CCR7 following ligation of CCL19 but not CCL21. Involved in internalization of P2RY1, P2RY4, P2RY6 and P2RY11 and ATP-stimulated internalization of P2RY2. Involved in phopshorylation-dependent internalization of OPRD1 and subsequent recycling or degradation. Involved in ubiquitination of IGF1R. Beta-arrestins function as multivalent adapter proteins that can switch the GPCR from a G-protein signaling mode that transmits short-lived signals from the plasma membrane via small molecule second messengers and ion channels to a beta-arrestin signaling mode that transmits a distinct set of signals that are initiated as the receptor internalizes and transits the intracellular compartment. Acts as signaling scaffold for MAPK pathways such as MAPK1/3 (ERK1/2) and MAPK10 (JNK3). ERK1/2 and JNK3 activated by the beta-arrestin scaffold are largely excluded from the nucleus and confined to cytoplasmic locations such as endocytic vesicles, also called beta-arrestin signalosomes. Acts as signaling scaffold for the AKT1 pathway. GPCRs for which the beta-arrestin-mediated signaling relies on both ARRB1 and ARRB2 (codependent regulation) include ADRB2, F2RL1 and PTH1R. For some GPCRs the beta-arrestin-mediated signaling relies on either ARRB1 or ARRB2 and is inhibited by the other respective beta-arrestin form (reciprocal regulation). Increases ERK1/2 signaling in AGTR1- and AVPR2-mediated activation (reciprocal regulation). Involved in CCR7-mediated ERK1/2 signaling involving ligand CCL19. Is involved in type-1A angiotensin II receptor/AGTR1-mediated ERK activity. Is involved in type-1A angiotensin II receptor/AGTR1-mediated MAPK10 activity. Is involved in dopamine-stimulated AKT1 activity in the striatum by disrupting the association of AKT1 with its negative regulator PP2A. Involved in AGTR1-mediated chemotaxis. Appears to function as signaling scaffold involved in regulation of MIP-1-beta-stimulated CCR5-dependent chemotaxis. Involved in attenuation of NF-kappa-B-dependent transcription in response to GPCR or cytokine stimulation by interacting with and stabilizing CHUK. Suppresses UV-induced NF-kappa-B-dependent activation by interacting with CHUK. The function is promoted by stimulation of ADRB2 and dephosphorylation of ARRB2. Involved in p53/TP53-mediated apoptosis by regulating MDM2 and reducing the MDM2-mediated degradation of p53/TP53. May serve as nuclear messenger for GPCRs. Upon stimulation of OR1D2, may be involved in regulation of gene expression during the early processes of fertilization. Also involved in regulation of receptors others than GPCRs. Involved in endocytosis of TGFBR2 and TGFBR3 and down-regulates TGF-beta signaling such as NF-kappa-B activation. Involved in endocytosis of low-density lipoprotein receptor/LDLR. Involved in endocytosis of smoothened homolog/Smo, which also requires ADRBK1. Involved in endocytosis of SLC9A5. Involved in endocytosis of ENG and subsequent TGF-beta-mediated ERK activation and migration of epithelial cells. Involved in Toll-like receptor and IL-1 receptor signaling through the interaction with TRAF6 which prevents TRAF6 autoubiquitination and oligomerization required for activation of NF-kappa-B and JUN. Involved in insulin resistence by acting as insulin-induced signaling scaffold for SRC, AKT1 and INSR. Involved in regulation of inhibitory signaling of natural killer cells by recruiting PTPN6 and PTPN11 to KIR2DL1.

Sequence similarities

Belongs to the arrestin family.

Domain

The [DE]-X(1,2)-F-X-X-[FL]-X-X-X-R motif mediates interaction the AP-2 complex subunit AP2B1.

Post-translational modifications

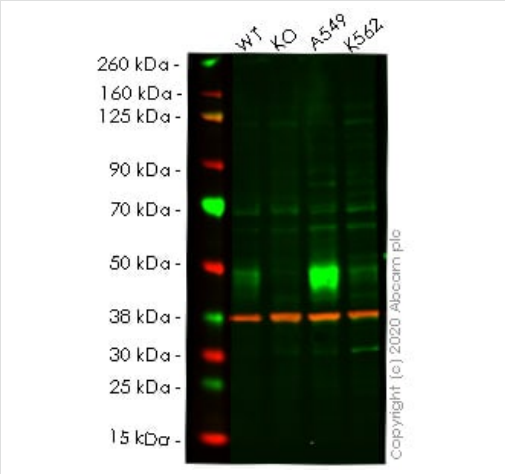
Phosphorylated at Thr-382 in the cytoplasm; probably dephosphorylated at the plasma membrane. The phosphorylation does not regulate internalization and recycling of ADRB2, interaction with clathrin or AP2B1.

The ubiquitination status appears to regulate the formation and trafficking of beta-arrestin-GPCR complexes and signaling. Ubiquitination appears to occurr GPCR-specifc. Ubiquitinated by MDM2; the ubiquitination is required for rapid internalization of ADRB2. Deubiquitinated by USP33; the deubiquitination leads to a dissociation of the beta-arrestin-GPCR complex. Stimulation of a class A GPCR, such as ADRB2, induces transient ubiquitination and subsequently promotes association with USP33. Stimulation of a class B GPCR promotes a

sustained ubiquitination.

Cellular localization	Cytoplasm. Nucleus. Cell membrane. Membrane > clathrin-coated pit. Cytoplasmic vesicle. Translocates to the plasma membrane and colocalizes with antagonist-stimulated GPCRs.

Images



Western blot - Anti-Beta Arrestin 2 antibody (ab54790)

All lanes : Anti-Beta Arrestin 2 antibody (ab54790) at 1/500 dilution

Lane 1 : Wild-type HepG2 cell lysate
Lane 2 : ARRB2 knockout HepG2 cell lysate
Lane 3 : A549 cell lysate
Lane 4 : K562 cell lysate

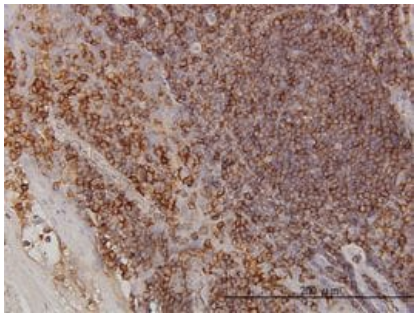
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 55 kDa
why is the actual band size different from the predicted?

Lanes 1- 4: Merged signal (red and green). Green - ab54790 observed at 55 kDa. Red - Anti-GAPDH antibody[EPR16891] - Loading Control (ab181602) observed at 37 kDa.

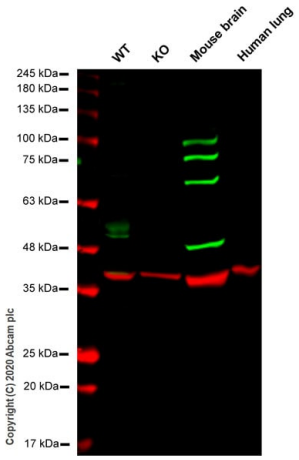
ab54790 was shown to react with ARRB2 in wild-type HepG2 cells in western blot. Loss of signal was observed when knockout cell line ab262320 (knockout cell lysate ab257283) was used. Wild-type HepG2 and ARRB2 knockout HepG2 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab54790 and Anti-GAPDH antibody[EPR16891] - Loading Control (ab181602) overnight at 4°C at a 1 in 500 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye®800CW) preadsorbed (ab216772) and Goat Anti-Rabbit IgG H&L (IRDye®680RD) preadsorbed (ab216777) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Beta Arrestin 2 antibody (ab54790)

Beta Arrestin 2 antibody (ab54790) used in immunohistochemistry at 3ug/ml on formalin fixed and paraffin embedded human tonsil.

This image was generated using the ascites version of the product.



Western blot - Anti-Beta Arrestin 2 antibody (ab54790)

All lanes : Anti-Beta Arrestin 2 antibody (ab54790) at 1/500 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : ARRB2 knockout HEK293T cell lysate
Lane 3 : Mouse brain tissue lysate
Lane 4 : Human lung tissue lysate

Lysates/proteins at 20 µg per lane.

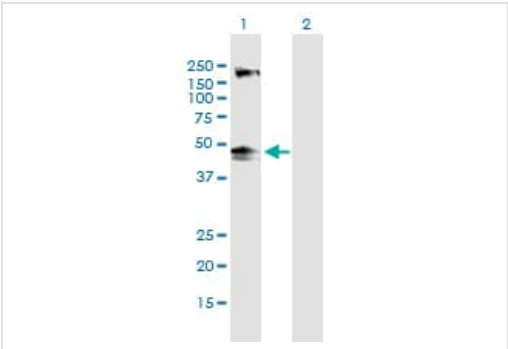
Performed under reducing conditions.

Observed band size: 55 kDa why is the actual band size different from the predicted?

Lanes 1-4: Merged signal (red and green). Green - ab54790 observed at 48-55 kDa. Red - loading control, ab181602 observed at 37 kDa.

ab54790 Anti-Beta Arrestin 2 antibody was shown to specifically react with Beta Arrestin 2 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266116 (knockout cell lysate ab257282) was used. Wild-type and Beta Arrestin 2 knockout samples were subjected to SDS-PAGE. ab54790 and Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse

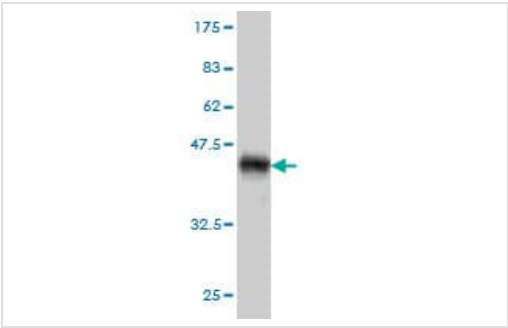
IgG H&L (IRDye® 800CW) preadsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Beta Arrestin 2 antibody (ab54790)

All lanes : Anti-Beta Arrestin 2 antibody (ab54790)

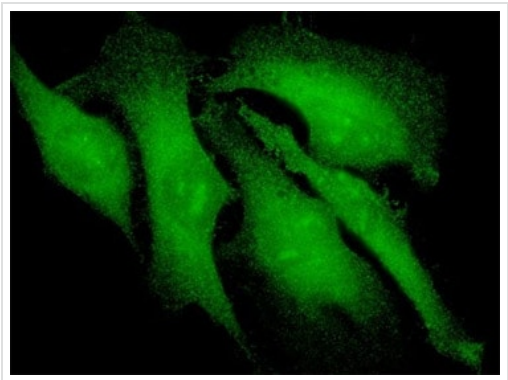
Lane 1 : Beta Arrestin 2 transfected 293T
Lane 2 : Non- transfected 293T



Western blot - Anti-Beta Arrestin 2 antibody (ab54790)

Western blot against tagged recombinant protein immunogen using ab54790 Beta Arrestin 2 antibody at 1ug/ml. Predicted band size of immunogen is 35 kDa

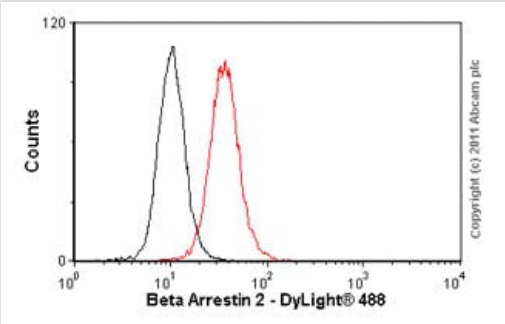
This image was generated using the ascites version of the product.



Immunocytochemistry/ Immunofluorescence - Anti-Beta Arrestin 2 antibody (ab54790)

ab54790 at 10 ug/ml staining Beta Arrestin 2 in human Hela cells by Immunocytochemistry / Immunofluorescence.

This image was generated using the ascites version of the product.



Flow Cytometry - Anti-Beta Arrestin 2 antibody (ab54790)

Overlay histogram showing HeLa cells stained with ab54790 (red line). The cells were fixed with 4% paraformaldehyde (10 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 (ab54790, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was a mix of mouse IgG2a [ICIGG2A], (ab91361, 1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 80% methanol/permeabilized in 0.1% PBS-Tween used under the same conditions.

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