abcam

Product datasheet

Anti-beta Arrestin 1 antibody [E274] - BSA and Azide free ab206776



8 References 9 Images

Overview

Product name Anti-beta Arrestin 1 antibody [E274] - BSA and Azide free

Description Rabbit monoclonal [E274] to beta Arrestin 1 - BSA and Azide free

Host species Rabbit

Specificity The antibody immunogen shares 90% homology with ARRB2 (P32121) which has similar MW

than ARRB1. Therefore, it is likely that the antbody will cross-react with ARRB2. However, we

haven't performed any experiment with recombinant protein to confirm this.

Tested applications Suitable for: Flow Cyt (Intra), IHC-P, WB, ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Cow

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control 293 cell lysate and human lymph node tissue.

General notes ab206776 is the carrier-free version of <u>ab32099</u>.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for

increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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

1

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. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal

Clone number E274

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab206776 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 (Intra)		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
WB		Use at an assay dependent concentration. Predicted molecular weight: 50 kDa.
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 receptorbound arrestin is removed so that the receptor can be dephosphorylated and returned to the plasma membrane. Involved in internalization of P2RY4 and UTP-stimulated internalization of P2RY2. Involved in phopshorylation-dependent internalization of OPRD1 ands subsequent recycling. Involved in the degradation of cAMP by recruiting cAMP phosphodiesterases to ligandactivated receptors. 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). ERK1/2 activated by the beta-arrestin scaffold is largely excluded from the nucleus and confined to cytoplasmic locations such as endocytic vesicles, also called betaarrestin signalosomes. Recruits c-Src/SRC to ADRB2 resulting in ERK activation. 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). Inhibits ERK1/2 signaling in AGTR1- and AVPR2-mediated activation (reciprocal regulation). Is required for SP-stimulated endocytosis of NK1R and recruits c-Src/SRC to internalized NK1R resulting in ERK1/2 activation, which is required for the antiapoptotic effects of SP. Is involved in proteinase-activated F2RL1-mediated ERK activity. Acts as signaling scaffold for the AKT1 pathway. Is involved in alpha-thrombin-stimulated AKT1 signaling. Is involved in IGF1-stimulated AKT1 signaling leading to increased protection from apoptosis. Involved in activation of the p38 MAPK signaling pathway and in actin bundle formation. Involved in F2RL1-mediated cytoskeletal rearrangement and chemotaxis. Involved in AGTR1-mediated stress fiber formation by acting together with GNAQ to activate RHOA. Appears to function as signaling scaffold involved in regulation of MIP-1-beta-stimulated CCR5dependent chemotaxis. Involved in attenuation of NF-kappa-B-dependent transcription in response to GPCR or cytokine stimulation by interacting with and stabilizing CHUK. May serve as nuclear messenger for GPCRs. Involved in OPRD1-stimulated transcriptional regulation by translocating to CDKN1B and FOS promoter regions and recruiting EP300 resulting in acetylation of histone H4. Involved in regulation of LEF1 transcriptional activity via interaction with DVL1 and/or DVL2 Also involved in regulation of receptors others than GPCRs. Involved in Tolllike 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. Binds phosphoinositides. Binds inositolhexakisphosphate (InsP6).

Sequence similarities

Domain

Belongs to the arrestin family.

The [DE]-X(1,2)-F-X-X-[FL]-X-X-R motif mediates interaction the AP-2 complex subunit AP2B1 (By similarity). Binding to phosphorylated GPCRs induces a conformationanl change that exposes the motif to the surface.

The N-terminus binds InsP6 with low affinity.

The C-terminus binds InsP6 with high affinity.

Post-translational modifications

Constitutively phosphorylated at Ser-412 in the cytoplasm. At the plasma membrane, is rapidly dephosphorylated, a process that is required for clathrin binding and ADRB2 endocytosis but not for ADRB2 binding and desensitization. Once internalized, is rephosphorylated.

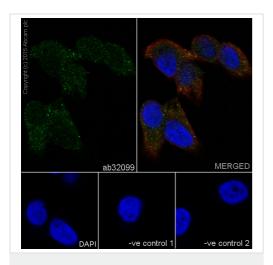
The ubiquitination status appears to regulate the formation and trafficking of beta-arrestin-GPCR

complexes and signaling. Ubiquitination appears to occur GPCR-specific. 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.

Cellular localization

Cytoplasm. Nucleus. Cell membrane. Membrane > clathrin-coated pit. Cell projection > pseudopodium. Cytoplasmic vesicle. Translocates to the plasma membrane and colocalizes with antagonist-stimulated GPCRs. The monomeric form is predominantly located in the nucleus. The oligomeric form is located in the cytoplasm. Translocates to the nucleus upon stimulation of OPRD1.

Images



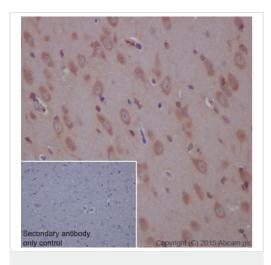
Immunocytochemistry/ Immunofluorescence - Antibeta Arrestin 1 antibody [E274] - BSA and Azide free (ab206776)

Immunocytochemistry/Immunofluorescence analysis of PC-3 cells labelling beta Arrestin 1 with purified **ab32099** at a dilution of 1/150. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000) were also used.

Control 1: primary antibody (1/150) and secondary antibody, **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: $\underline{ab7291}$ (1/1000) and secondary antibody, $\underline{ab150077}$, an Alexa Fluor[®] 488-conjugated goat anti-rabbit lgG (1/1000).

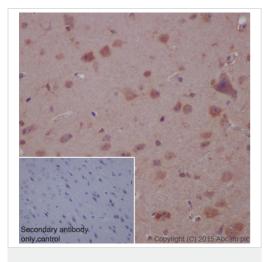
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32099).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta Arrestin 1 antibody
[E274] - BSA and Azide free (ab206776)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat cerebral cortex tissue labelling beta Arrestin 1 with purified ab32099 at a dilution of 1/250. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

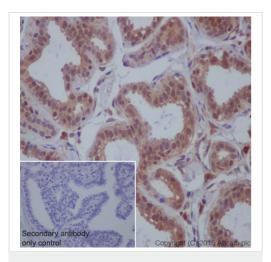
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32099).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta Arrestin 1 antibody [E274] - BSA and Azide free (ab206776)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse cerebral cortex tissue labelling beta Arrestin 1 with purified ab32099 at a dilution of 1/250. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

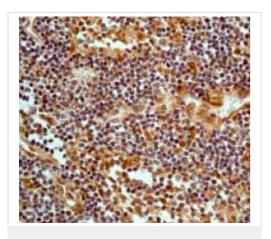
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab32099</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta Arrestin 1 antibody
[E274] - BSA and Azide free (ab206776)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labelling beta Arrestin 1 with purified <u>ab32099</u> at a dilution of 1/250. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. <u>ab97051</u>, a HRP-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32099).

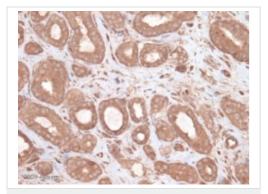


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta Arrestin 1 antibody [E274] - BSA and Azide free (ab206776)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lymph node tissue labelling beta Arrestin 1 with unpurified **ab32099** at a dilution of 1/100.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32099).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

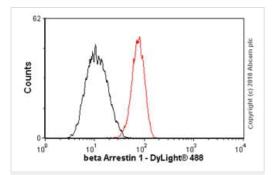


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-beta Arrestin 1 antibody [E274] - BSA and Azide free (ab206776)

This image is courtesy of an anonymous Abreview.

Unpurified <u>ab32099</u> staining beta Arrestin 1 in human prostate carcinoma tissue section by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue underwent paraformaldehyde fixation before heat mediated antigen retrieval in Tris/EDTA pH9.0 and then blocking with 1% donkey serum for 1 hour at 20°C was performed. The primary antibody was diluted 1/100 and incubated with sample for 1 hour at 20°C in PBS. A Biotin conjugated donkey polyclonal to rabbit lgG was used undiluted as secondary antibody.

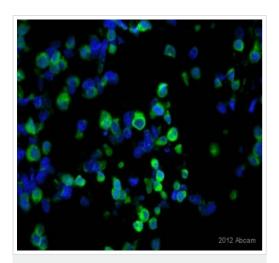
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32099).



Flow Cytometry (Intracellular) - Anti-beta Arrestin 1 antibody [E274] - BSA and Azide free (ab206776)

Overlay histogram showing PC3 cells stained with unpurified ab32099 (red line). The cells were fixed with 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 (unpurified ab32099, 1/10 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal lgG (1µg/1x106 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a decreased signal in PC3 cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32099).



Immunocytochemistry/ Immunofluorescence - Antibeta Arrestin 1 antibody [E274] - BSA and Azide free (ab206776)

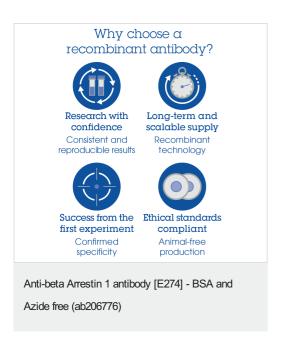
This image is courtesy of an anonymous Abreview.

Unpurified <u>ab32099</u> staining beta Arrestin 1 in C4-2B (Human prostate cancer cell line) by ICC/IF

(Immunocytochemistry/immunofluorescence).

Cells were fixed with paraformaldehyde and blocked with 1% serum for 1 hour at 21°C. Samples were incubated with primary antibody (1/100 in diluent) for 1 hour at 21°C. An Alexa Fluor[®] 488-conjugated goat anti-rabbit polyclonal IgG (1/200) was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32099).



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors