abcam

Product datasheet

Anti-p115-RhoGEF antibody [JH-1] ab243248

Recombinant

7 Images

Overview

Product name Anti-p115-RhoGEF antibody [JH-1]

Description Armenian hamster monoclonal [JH-1] to p115-RhoGEF

Host species Armenian hamster

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

Unsuitable for: Flow Cyt

Species reactivity Reacts with: Mouse, Rat

Immunogen Recombinant fragment within Mouse p115-RhoGEF. The exact immunogen sequence used to

generate this antibody is proprietary information. If additional detail on the immunogen is needed to determine the suitability of the antibody for your needs, please **contact** our Scientific Support

team to discuss your requirements.

Database link: Q61210

Run BLAST with
Run BLAST with

Positive control WB: A20, EL4, PC-12 and C6 whole cell lysates. ICC: A20, Neuro2a and EL4 cells. Flow Cyt

(intra): EL4 cells.

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.

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

For more information see here.

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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)

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Purity Protein A purified

Clonality Monoclonal

Clone number JH-1 lsotype lgG

Applications

The Abpromise guarantee

Our Abpromise quarantee covers the use of ab243248 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)		1/40.
WB		1/1000. Detects a band of approximately 115 kDa (predicted molecular weight: 102 kDa).
ICC/IF		1/50.
ICC		1/50.

Application notes Is unsuitable for Flow Cyt.

Target	
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Function Seems to play a role in the regulation of RhoA GTPase by guanine nucleotide-binding alpha-12

(GNA12) and alpha-13 (GNA13) subunits. Acts as GTPase-activating protein (GAP) for GNA12 and GNA13, and as guanine nucleotide exchange factor (GEF) for RhoA GTPase. Activated G alpha 13/GNA13 stimulates the RhoGEF activity through interaction with the RGS-like domain. This GEF activity is inhibited by binding to activated GNA12. Mediates angiotensin-2-induced

RhoA activation.

Tissue specificity Ubiquitously expressed.

Sequence similarities Contains 1 DH (DBL-homology) domain.

Contains 1 PH domain.

Contains 1 RGSL (RGS-like) domain.

Domain The RGSL domain, also known as rgRGS domain, is necessary but not sufficient for GAP activity.

The DH domain is involved in interaction with CCPG1.

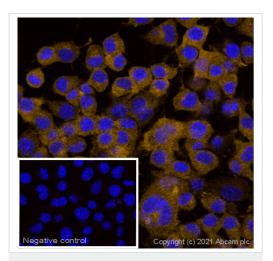
Post-translational modifications

Phosphorylated by PKCA. Angiotensin-2 induced Tyr-738 phosphorylation is mediated by JAK2.

Cellular localization

Cytoplasm. Membrane. Translocated to the membrane by activated GNA13 or LPA stimulation.

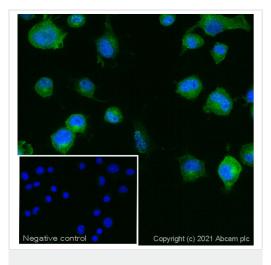
Images



Immunocytochemistry/ Immunofluorescence - Antip115-RhoGEF antibody [JH-1] (ab243248)

ICC/IF image of ab243248 stained Neuro2a cells. The cells were 4% formaldehyde fixed (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to block nonspecific protein-protein interactions. The cells were then incubated with the antibody (ab243248, 1.6μg/ml) overnight at +4°C. The secondary antibody (orange) was ab175716 Goat Anti-Armenian hamster IgG H&L (Alexa Fluor® 568) used at 1μg/ml for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43μM.

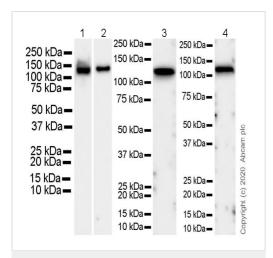
The negative control (inset) is a secondary-only assay to demonstrate low non-specific binding of the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Antip115-RhoGEF antibody [JH-1] (ab243248)

ICC/IF image of ab243248 stained Neuro2a cells. The cells were 4% formaldehyde fixed (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab243248, 1.6µg/ml) overnight at +4°C. The secondary antibody (green) was $\underline{ab173003}$ Goat Anti-Armenian hamster $\lg G$ H&L (Alexa Fluor® 488) used at 1µg/ml for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

The negative control (inset) is a secondary-only assay to demonstrate low non-specific binding of the secondary antibody.



Western blot - Anti-p115-RhoGEF antibody [JH-1] (ab243248)

All lanes : Anti-p115-RhoGEF antibody [JH-1] (ab243248) at 1/1000 dilution

Lane 1 : A20 (mouse reticulum sarcoma B lymphocyte), whole cell lysate

Lane 2: EL4 (mouse lymphoma T lymphocyte), whole cell lysate

Lane 3: PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate

Lane 4: C6 (rat glial tumor glial cell), whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Rabbit Anti-Armenian hamster lgG H&L (HRP) (<u>ab5745</u>) at 1/5000 dilution

Predicted band size: 102 kDa **Observed band size:** 115 kDa

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 11384980).

Lysates should be made freshly and used in WB immediately.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lanes 1/2:3 seconds; Lane 3:15 seconds; Lane 4:37 seconds.

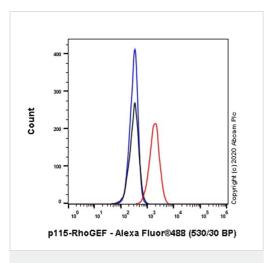
ab243248 MERGED

Immunocytochemistry/ Immunofluorescence - Antip115-RhoGEF antibody [JH-1] (ab243248)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A20 (mouse reticulum sarcoma B lymphocyte) cells labelling p115-RhoGEF with ab243248 at 1/50 dilution, followed by ab173003 Goat Anti-Armenian hamster lgG H&L (Alexa Fluor[®] 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in A20 cells. ab179513 Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/200 dilution, followed by ab150080 Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 594) at a 1/1000 dilution (Red). The nuclear counterstain was DAPI (Blue).

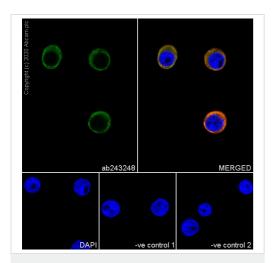
Negative control 1: ab243248 at a 1/50 dilution followed by ab150080 at a 1/1000 dilution.

Negative control 2: <u>ab179513</u> at a 1/200 dilution followed by <u>ab173003</u> at a 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-p115-RhoGEF antibody [JH-1] (ab243248)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized EL-4 (mouse lymphoma T lymphocyte) cells labelling p115-RhoGEF with ab243248 at 1/40 dilution (1µg) (Red) compared with an Armenian hamster monoclonal lgG (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti armenian hamster lgG (Alexa Fluor® 488, ab173003) at 1/2000 dilution was used as the secondary antibody.

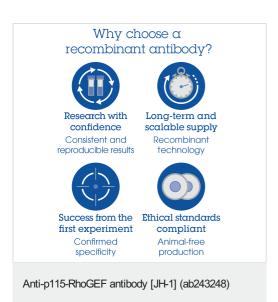


Immunocytochemistry/ Immunofluorescence - Antip115-RhoGEF antibody [JH-1] (ab243248)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized EL4 (mouse lymphoma T lymphocyte) cells labelling p115-RhoGEF with ab243248 at 1/50 dilution, followed by ab173003 Goat Anti-Armenian hamster lgG H&L (Alexa Fluor[®] 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in EL4 cells. ab179513 Anti-beta Tubulin rabbit monoclonal antibody was used to counterstain tubulin at 1/200 dilution, followed by ab150080 Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 594) at a 1/1000 dilution (Red). The nuclear counterstain was DAPI (Blue).

Negative control 1: ab243248 at a 1/50 dilution followed by **ab150080** at a 1/1000 dilution.

Negative control 2: <u>ab179513</u> at a 1/200 dilution followed by ab173003 at a 1/1000 dilution.



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