Datasheet for ABIN110590
anti-Ga16 antibody

1 Validation

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Overview

Quantity:	0.2 mg
Target:	Ga16
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	Un-conjugated
Application:	Immunoprecipitation (IP), Western Blotting (WB)

Product Details

Target Details

Target:	Ga16
Background:	Ga 16 is the only hetero-trimeric G proteins with a restricted expression pattern in hematopoietic cells. Differentiation of promyelocyticcells leads to decreased expression of Ga 16. This G protein is known to couple a large number of G protein-coupled receptors to PLC-ß and can lead to production of the secondary messengers diacylglycerol and inositol phosphates. Targeted deletion of Ga 15, a mouse orthologue of human Ga 16, causes reduced C5a receptor signaling. Ga 16serves as a marker, in addition to CD34, for hematopoietic progenitor cells.

Application Details

Restrictions:	For Research Use only

Handling

Format:	Lyophilized
Storage:	4 °C

Validation report #100205 for Western Blotting (WB)

Successfully validated (Western Blotting (WB))

by Laboratorio Universitario di Ricerca Medica, Univsersità degli Studi di Verona

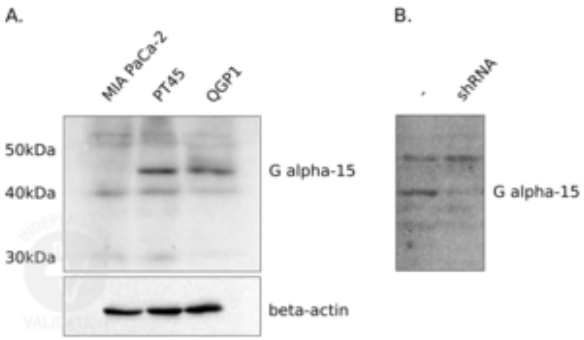
Report Number: 100205

Date: Nov 27 2016

Target:	Ga16
Lot Number:	82100
Method validated:	Western Blotting (WB)
Positive Control:	QGP1 and PT45 lysates
Negative Control:	MIA PaCa-2 cells lysate; PT45 shRNA knock-down cell lysate
Primary Antibody:	ABIN110590
Secondary Antibody:	goat anti-rabbit IgG HRP antibody (Sigma-Aldrich, A0545)
Protocol:	• Pancreatic cancer cells lines QGP1, PT45, and MIA PaCa-2 were grown in DMEM 10% FBS at 37°C in 5% CO_2. • PT45 cells were transduced with lentiviral particles to express shRNA that specifically targets G alpha-15 mRNA. • Wash cells with PBS. • Collect and lyse cells in RIPA buffer supplemented with protease inhibitor cocktail. • Determine total protein content of the lysates using bicinchoninic acid (BCA) method. • Adjust samples to 1µg/µl protein using RIPA buffer and Laemmli buffer. • Boil samples for 5min and separate 20µg of each sample on a 10% acrylamide gel at 100V until the salt/dye front reached the bottom. • Transfer proteins onto PVDF membrane. • Block the membrane with TBST containing 5% milk for 1 h at room temperature. • Incubation with primary GNA15 antibody (antibodies-online, ABIN110590, lot 82100) diluted 1:500 in TBST 5% milk for 2h at RT. • Wash membrane 4x 5min in TBST. • Incubation with secondary goat anti-rabbit IgG HRP antibody (Sigma-Aldrich, A0545) 1:10000. • Wash membrane 4x 5 minutes in TBST. • Reveal protein bands using Luminata Forte Western HRP substrate (Millipore, WBLUF0500) as substrate and Syngene G-box to image light emission. • Strip membranes (2% SDS, 62.5mM TrisHCl pH6.8, 0.8% beta-mercaptoethanol) and subsequently incubate with a beta-actin loading control antibody (Santa Cruz clone (C4), sc-47778, lot D0108) diluted 1:1000 and secondary anti-mouse IgG HRP antibody (Sigma-Aldrich, A2554) diluted 1:10000.

	• Wash and reveal the protein as described above.
Experimental Notes:	Passed. The inhibition proves that the antibody is specific for GNA15 and it does not recognize one of the more abundant and ubiquitous homologs, GNAQ or GNA11.

Image for Validation report #100205



Validation image no. 1 for anti-Ga16 antibody (ABIN110590)

Immunoblot in TBST using milk 5% as blocking agent; see protocol for details. A. Lysate from pancreatic cancer cell lines was loaded as indicated. B. Lysate from PT45 cells, untreated in lane 1 and treated with shRNA specific for GNA15 in lane 2.