Datasheet for ABIN1533291

anti-Metabotropic Glutamate Receptor 6 antibody (AA 828-877)

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Overview

Quantity:	100 µg
Target:	Metabotropic Glutamate Receptor 6 (GRM6)
Binding Specificity:	AA 828-877
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This Metabotropic Glutamate Receptor 6 antibody is un-conjugated
Application:	Western Blotting (WB), ELISA, Immunohistochemistry (IHC)

Product Details

Immunogen:	The antiserum was produced against synthesized peptide derived from human mGluR6.
Isotype:	IgG
Specificity:	mGluR6 Antibody detects endogenous levels of total mGluR6 protein.
Purification:	The antibody was purified from rabbit antiserum by affinity-chromatography using immunogen.
Purity:	> 95 %

Target Details

Target:	Metabotropic Glutamate Receptor 6 (GRM6)
Alternative Name:	mGluR6 (GRM6 Products)
Background:	Synonyms: CSNB1B, Glu6, glutamate receptor, metabotropic 6, GPRC1F, metabotropic

Target Details

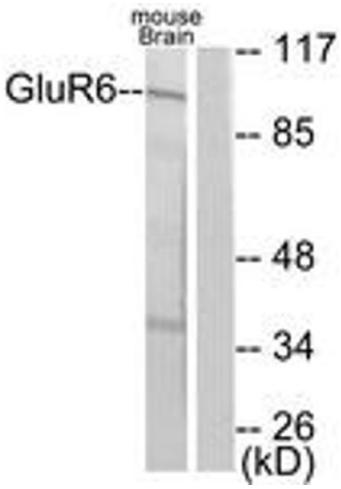
	glutamate receptor 6 NCBI Gene Symbol: GRM6
Molecular Weight:	95 kDa
Gene ID:	2916
OMIM:	257270
UniProt:	O15303

Application Details

Application Notes:	WB: 1:500~1:1000 IHC: 1:50~1:100 ELISA: 1:5000
Comment:	Unigene-Number: Hs.248131 (NCBI Gene Symbol: GRM6)
Restrictions:	For Research Use only

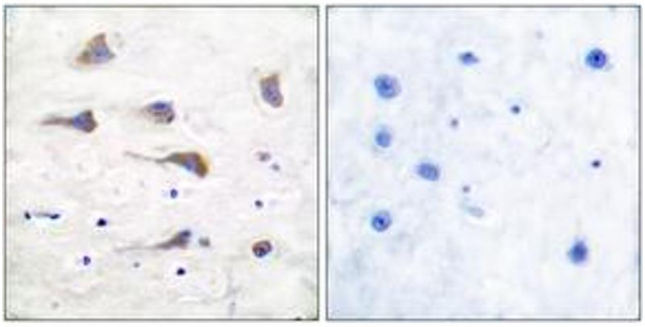
Handling

Format:	Liquid
Concentration:	1 mg/mL
Buffer:	phosphate buffered saline (without Mg2+ and Ca2+), pH 7.4, 150 mM NaCl, 0.02 % sodium azide and 50 % glycerol.
Preservative:	Sodium azide
Precaution of Use:	This product contains sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C
Storage Comment:	Stable at -20°C for at least 1 year.
Expiry Date:	12 months



Western Blotting

Image 1. Western blot analysis of extracts from mouse brain cells, using mGluR6 Antibody. The lane on the right is treated with the synthesized peptide.



Immunohistochemistry

Image 2. Immunohistochemistry analysis of paraffin-embedded human brain tissue, using mGluR6 Antibody. The picture on the right is treated with the synthesized peptide.

Validation report #103490 for Western Blotting (WB)

Successfully validated (Western Blotting (WB))

by Group Kleinlogel, Department of Physiology, University of Bern, Bern, Switzerland

Report Number: 103490

Date: Mar 15 2019

Target:	GRM6
Lot Number:	310210
Method validated:	Western Blotting (WB)
Positive Control:	Chinese Hamster Ovary (CHO-K1) cells transfected with a plasmid expressing human GRM6
Negative Control:	Non-transfected CHO-K1 cells
Notes:	Passed. ABIN1533291 specifically recognizes human GRM6 expressed in CHO cells.
Primary Antibody:	ABIN1533291
Secondary Antibody:	HRP conjugated goat anti-rabbit antibody (Jackson Immuno Research, 111-035-144)
Protocol:	• Grow Chinese Hamster Ovary (CHO-K1) cells (ECACC) in DMEM (Sigma, D5671, lot RNB68272) supplemented with 10% Fetal calf Serum (Seraglob, S70500, lot 208/203142), 1x MEM Non-Essential-Amino-Acid (Sigma, M7145, lot RNBC8122), L-Alanyl-L-Glutammine (Merck, K0302) and Pen/Strep (Sigma, P4333, lot 058M4857V), at 37°C and 5% CO_2. • Plate 0.25x10^6cells/ml in 2ml/well of cells in a 6 well plate. • Grow cells for 24h at 37°C and 5% CO_2. • Transfect cells with 2ug/well of a plasmid expressing human GRM6 under the control of the ubiquitous promoter CMV using TransIt-LT1 transfection reagent (Mirus, Mir2300, lot 81104333) following the manufacturer´s instructions. • Grow cells for 72h at 37°C and 5% CO_2. • Harvest cells with PBS and lyse them on ice for 30min with 50µl/well of RIPA buffer (25mM TrispH7-8, 150mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% Triton X-100 or NP-40). • Complete the lysis with a freeze/taw cycle. • Determine total protein content of the lysates using Pierce BCA protein assay (Thermo Fisher, 23221). • Denature 25µg of total protein for 20min at 37°C in 20µl Laemmli SDS sample buffer and subsequently separate them on a denaturing 4-20% Mini-PROTEAN TGX Stain-Free Gel (Bio-Rad, 456-8094) for 20min at 100V and 2h at 130V. • Transfer proteins onto Immobilion-P transfer membrane (Immobilion, IPVH00010) for 75min at 100mA. • Block the membrane with TBST 5% milk for 1h at RT. • Incubate with primary

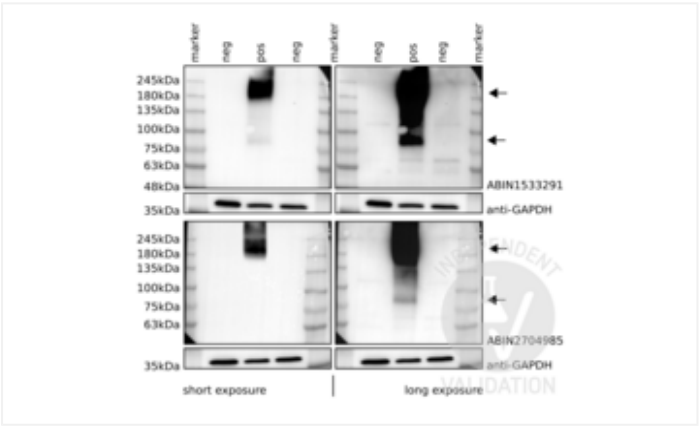
- rabbit anti-GRM6 antibody (antibodies-online, ABIN1533291, lot 310210) diluted 1:1000 in TBST 5% milk ON at 4°C,
- rabbit anti-GRM6 antibody (antibodies-online, ABIN2704985, lot 73482) diluted 1:1000 (positive control), or
- mouse anti-GAPDH antibody (Fitzgerald, 10R-G09a, lot 2417) diluted 1:40000 in TBST 5% milk ON at 4°C.

- Wash membrane 3x for 5min with TBST buffer.
- Incubation with secondary
  - HRP conjugated goat anti-rabbit antibody (Jackson Immuno Research, 111-035-144) diluted 1:3000 in TBST 5% milk for 45min at RT or
  - goat anti-mouse antibodies (Jackson Immuno Research, 115-035-146) diluted 1:3000 in TBST 5% milk for 45min at RT.
- Wash membrane 3x for 10min with TBST buffer.
- Reveal protein bands using Clarity MAX Western ECL Substrate (Bio-Rad, 1705062) on a ChemiDoc MP imaging system (Bio-Rad, 17001402).

Experimental Notes:

- ABIN1533291 reveals two distinct bands. A lower one, which molecular weight corresponds to the monomer of the target protein, and a higher one which appears to correspond to a dimer of GRM6. The protein bands are only visible in the positive but not in the negative controls. Importantly, the same result was observed with another anti GRM6 antibody (ABIN2704985).
- Being a G-Protein-Coupled Receptor it is not surprising that GRM6 forms strong dimers that can withstand boiling. Several attempts were performed in order to dissolve such dimers including sonication and different boiling protocols but they were unsuccessful. Similar results were obtained with HEK293 cells as well (not shown).

Image for Validation report #103490



Validation image no. 1 for anti-Glutamate Receptor, Metabotropic 6 (GRM6) (AA 828-877) antibody (ABIN1533291)

Western blot analysis of cell lysates from CHO-K1 cells transfected with a human GRM6 expression plasmid (pos) or untransfected cells (neg) using ABIN1533291 (top), ABIN2704985 (bottom) with short or long exposure times, or an anti-GAPDH loading control antibody. Arrows indicate what appear to be GRM6 monomers and dimers.

Successfully validated (Immunofluorescence (IF))

by Group Kleinlogel, Department of Physiology, University of Bern, Bern, Switzerland

Report Number: 103795

Date: Mar 15 2019

Target:	GRM6
Lot Number:	310210
Method validated:	Immunofluorescence (IF)
Positive Control:	Chinese Hamster Ovary (CHO-K1) cells transfected with a plasmid expressing human GRM6 and turbo Red Fluorescent protein (tRFP)
Negative Control:	Non-transfected CHO-K1 cells CHO transfected with a plasmid expressing human GRM6 stained with secondary antibody only
Notes:	Passed. ABIN1533291 specifically labels the targeted antigen in transfected CHO-K1 cells in IF as demonstrated by the co-labeling with tRFP. No specific signal was detected in the negative controls.
Primary Antibody:	ABIN1533291
Secondary Antibody:	AF488 conjugated goat anti-rabbit IgG antibody (Invitrogen, A11008)

Protocol:	• Grow cells:
	  ◦ Grow Chinese Hamster Ovary (CHO-K1) cells (ECACC) in DMEM (Sigma, D5671, lot RNB68272) supplemented with 10% Fetal calf Serum (Seraglob, S70500, lot 208/203142), 1x MEM Non-Essential-Amino-Acid (Sigma, M7145, lot RNBC8122), L-Alanyl-L-Glutammine (Merck, K0302) and Penicillin/Streptomycin (Sigma, P4333, lot 058M4857V) at 37°C and 5% CO_2.
	  ◦ Plate 0.125x10^6cells/well in 0.5 ml/well in a 24 well plates on coverslips.
	  ◦ Grow cells for 24h at 37°C and 5% CO_2.
	• Transfect cells with a plasmid expressing human GRM6-tRFP under the control of the CMV promoter using Polyethylenimine hydrochloride (PEI) reagent (Sigma, 764965):
	  ◦ Add to 50µl of serum free Opti-MEM medium (Life Technologies, 31985-047) 0.5µg of DNA and 1.5µl of PEI.
	  ◦ Add the mixture directly into the wells and incubate for 4-5h.
	  ◦ Replace medium with fresh culture medium.
	  ◦ Grow cells for 72h at 37°C and 5% CO_2.
	• Fixation:
	  ◦ Fix cells in PFA 4% in PBS for 7-8min.

- Rinse 3x with 500µl 1x PBS.
- Add 200µl 0.1M Glycine in TBS for 15min.
- Remove Glycine with a pipette.
- Add blocking solution (5% Serum (NDS or NGS) + 1% BSA Serum in 0.3% Triton-X100 in 1x TBS) for 1h at RT.

- Primary antibody:
  - Dilute primary rabbit anti-GRM6 antibody (antibodies-online, ABIN1533291, lot 310210) 1:100 in blocking solution.
  - Spin the tube at 16000rcf for 6min to precipitate eventual particulate.
  - Add 200µl primary antibody solution per well.
  - Incubate slides in the dark ON at 4 °C.
  - Wash slides 3x 10min with 500µl 0.3% Triton-X100 in 1x TBS.
- Secondary antibody:
  - Dilute secondary AF488 conjugated goat anti-rabbit IgG antibody (Invitrogen, A11008) 1:400 and DAPI 1:2000 from a 1mg/ml stock dilution in blocking solution.
  - Spin the tube at 16000rcf for 6min to precipitate eventual particulate.
  - Add 200µl secondary antibody solution per well.
  - Incubate slides in the dark for 1-2 h at RT.
  - Wash slides 3x 10min with 500µl 0.3% Triton-X100 in 1x TBS.
- Mount slides:
  - Mount slides with fluorescence mounting medium (DAKO, S3023, lot 10115314).
  - Place a coverslip on it avoiding bubbles. Seal the edges of the coverslip with a clear nail polish.
  - Let the nail polish dry and store samples at -4°C in the dark.
- Microscopy:
  - Image acquisition on Zeiss LSM with a 20x objective. Gain(Master) of approximately 500.
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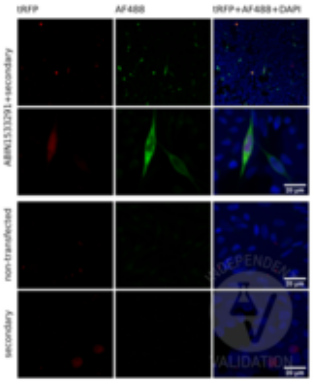
Image for Validation report #103795



Validation image no. 1 for anti-Glutamate Receptor, Metabotropic 6 (GRM6) (AA 828-877) antibody (ABIN1533291)

Low and high magnification pictures of CHO-K1 cells, transfected with a plasmid expressing human GRM6 and turbo Red Fluorescent protein (tRFP) stained with ABIN1533291 and an AF488 conjugated secondary antibody (AF488). Co-labeling of tRFP and GRM6 indicates the specificity of the antibody. Non-transfected cells (non-transfected) and transfected cells stained with the

secondary antibody only (secondary) served as negative controls.