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Datasheet for ABIN7295830

## anti-Metabotropic Glutamate Receptor 6 antibody (C-Term)

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### Overview

Quantity:	100 µL
Target:	Metabotropic Glutamate Receptor 6 (GRM6)
Binding Specificity:	C-Term
Reactivity:	Human, Rat, Mouse
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This Metabotropic Glutamate Receptor 6 antibody is un-conjugated
Application:	Western Blotting (WB), Immunofluorescence (IF), Immunochromatography (IC)

### Product Details

Immunogen:	KLH-conjugated synthetic peptide encompassing a sequence within the C-term region of human mGLUR6.
Specificity:	Recognizes endogenous levels of mGLUR6 protein.
Characteristics:	Rabbit polyclonal antibody to mGLUR6
Purification:	The antibody was purified by immunogen affinity chromatography.

### Target Details

Target:	Metabotropic Glutamate Receptor 6 (GRM6)
Alternative Name:	mGLUR6 ( <a href="#">GRM6 Products</a> )
Background:	GPRC1F, MGLUR6, Metabotropic glutamate receptor 6, mGluR6

## Target Details

Gene ID: 2916, 108072, 24419

UniProt: [O15303](#), [Q5NCH9](#), [P35349](#)

## Application Details

Application Notes: WB (1:500 - 1:1000), IF/IC (1:100 - 1:500)

Restrictions: For Research Use only

## Handling

Format: Liquid

Buffer: Liquid in 0.42 % Potassium phosphate, 0.87 % Sodium chloride, pH 7.3, 30 % glycerol, and 0.01 % sodium azide.

Preservative: Sodium azide

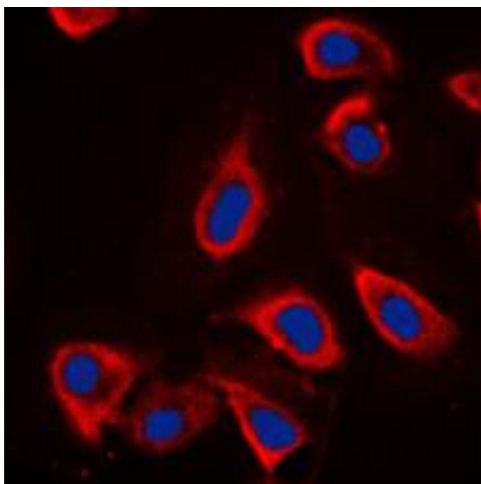
Precaution of Use: This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.

Storage: -20 °C

Storage Comment: Shipped at 4°C. Upon delivery aliquot and store at -20°C for one year. Avoid freeze/thaw cycles.

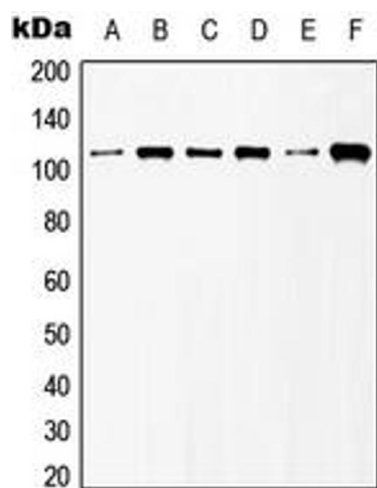
Expiry Date: 12 months

## Validation report #103814 for Western Blotting (WB)



### Immunofluorescence

**Image 1.** Immunofluorescent analysis of mGLUR6 staining in NIH3T3 cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with the primary antibody in 3% BSA-PBS and incubated overnight at 4 °C in a humidified chamber. Cells were washed with PBST and incubated with a DyLight 594-conjugated secondary antibody (red) in PBS at room temperature in the dark. DAPI was used to stain the cell nuclei (blue).



### Western Blotting

**Image 2.** Western blot analysis of mGLUR6 expression in HeLa (A), NIH3T3 (B), Raw264.7 (C), H9C2 (D), mouse brain (E), Jurkat (F) whole cell lysates.



### Successfully validated (Western Blotting (WB))

by [Group Kleinlogel, Department of Physiology, University of Bern, Bern, Switzerland](#)

Report Number: 103814

Date: Mar 15 2019

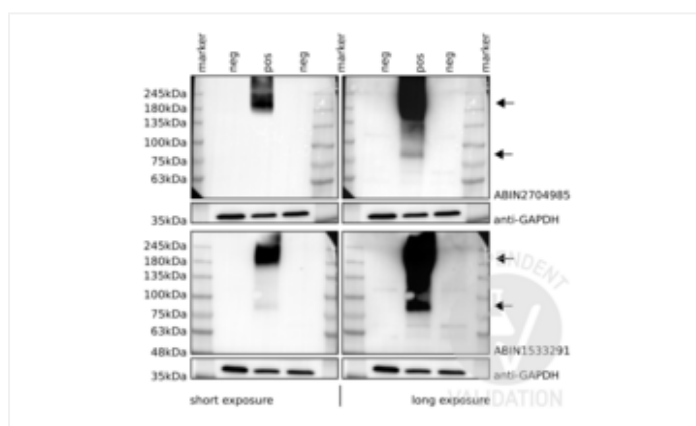
Target:	GRM6
Lot Number:	73482
Method validated:	Western Blotting (WB)
Positive Control:	Chinese Hamster Ovary (CHO-K1) cells transfected with a plasmid expressing human GRM6
Negative Control:	Non-transfected CHO-K1 cells
Notes:	Passed. ABIN2704985 specifically recognizes human GRM6 expressed in CHO cells.
Primary Antibody:	ABIN2704985
Secondary Antibody:	HRP conjugated goat anti-rabbit antibody (Jackson Immuno Research, 111-035-144)
Protocol:	<ul style="list-style-type: none"><li>• 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-Glutamine (Merck, K0302) and Pen/Strep (Sigma, P4333, lot 058M4857V), at 37°C and 5% CO<sub>2</sub>.</li><li>• Plate 0.25x10<sup>6</sup> cells/ml in 2ml/well of cells in a 6 well plate.</li><li>• Grow cells for 24h at 37°C and 5% CO<sub>2</sub>.</li><li>• 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.</li><li>• Grow cells for 72h at 37°C and 5% CO<sub>2</sub>.</li><li>• Harvest cells with PBS and lyse them on ice for 30min with 50µl/well of RIPA buffer (25mM Tris pH7-8, 150mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% Triton X-100 or NP-40).</li><li>• Complete the lysis with a freeze/thaw cycle.</li><li>• Determine total protein content of the lysates using Pierce BCA protein assay (Thermo Fisher, 23221).</li><li>• 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.</li><li>• Transfer proteins onto Immobilon-P transfer membrane (Immobilion, IPVH00010) for 75min at 100mA.</li><li>• Block the membrane with TBST 5% milk for 1h at RT.</li><li>• Incubate with primary</li></ul>

- rabbit anti-GRM6 antibody (antibodies-online, ABIN2704985, lot 73482) diluted 1:1000 (positive control),
- rabbit anti-GRM6 antibody (antibodies-online, ABIN1533291, lot 310210) diluted 1:1000 in TBST 5% milk ON at 4°C, 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:

- ABIN2704985 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 (ABIN1533291).
- 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 #103814



**Validation image no. 1 for anti-Glutamate Receptor, Metabotropic 6 (GRM6) (C-Term) antibody (ABIN2704985)**

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