

Datasheet for ABIN2690503

**anti-SLC1A3 antibody (Cytoplasmic Domain)**[Go to Product page](#)**1** Validation**3** Images

## Overview

Quantity:	50 µg
Target:	SLC1A3
Binding Specificity:	Cytoplasmic Domain
Reactivity:	Human, Rat, Mouse
Host:	Rabbit
Clonality:	Polyclonal
Application:	Western Blotting (WB), Immunohistochemistry (IHC), Immunocytochemistry (ICC)

## Product Details

Purification:	affinity purified
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## Target Details

Target:	SLC1A3
Alternative Name:	EAAT 1 ( <a href="#">SLC1A3 Products</a> )
Background:	Synonyms: GLAST, SLC1A3
Pathways:	<a href="#">Sensory Perception of Sound</a> , <a href="#">Synaptic Membrane</a> , <a href="#">Dicarboxylic Acid Transport</a>

## Application Details

Application Notes:	Optimal working dilution should be determined by the investigator. This product is not tested in IP yet.
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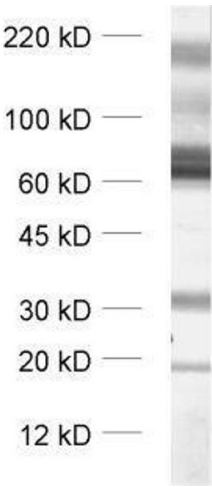
## Application Details

Restrictions: For Research Use only

## Handling

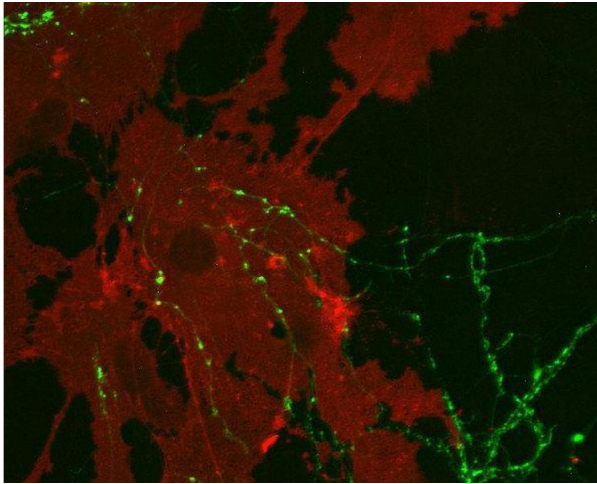
Format:	Lyophilized
Reconstitution:	For reconstitution add 200 µL H <sub>2</sub> O, then aliquot and store at -20 °C until use.
Buffer:	PBS
Handling Advice:	Affinity purified antibodies are less robust than antisera, since protease inhibitors are also removed during purification. Hence, storage at 4 °C for prolonged periods (i.e. several weeks), is not recommended.
Storage:	-20 °C
Storage Comment:	Unlabeled lyophilized antibodies are stable in this form without loss of quality at ambient temperatures for several weeks or even months. They can be stored at 4°C for several years. Lyophilized antibodies must not be stored in the freezer, they may be destroyed!

## Images



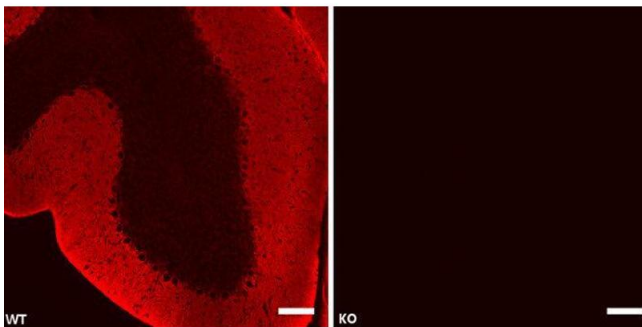
### Western Blotting

**Image 1.** dilution: 1 : 1000, sample: synaptic membrane fraction (LP1) of rat brain



#### Immunocytochemistry

**Image 2.** Indirect immunostaining of cultured rat astrocytes and hippocampus neurons with anti-EAAT 1 (dilution 1 : 1000; red) and mouse anti-synapsin 1 (cat. no. 106 001, dilution 1 : 500; green).



#### Immunohistochemistry

**Image 3.** Indirect immunostaining of EAAT 1 in mouse cerebellum of wildtype (wt) and knockout (ko) animals (red; dilution 1 : 5000).



## Successfully validated (Western Blotting (WB))

by [Department of Chemical Biology, Rutgers University](#)

Report Number: 103349

Date: Aug 29 2018

Target:	SLC1A3
Lot Number:	250113/3
Method validated:	Western Blotting (WB)
Positive Control:	Sut2 (xCT <sup>-/-</sup> ), MASS20 (xCT <sup>+/+</sup> ) and MelanA (xCT <sup>+/+</sup> ) cells are all mouse melanocytes that express SLC1A3 transporter. Sut2 and MelanA were from Dr. D. Bennett in 1998 and have kept in our lab since then, MASS20 was made in our lab.
Notes:	Passed. ABIN2690503 does specifically recognize SLC1A3 in all three tested murine melanocyte extracts.
Primary Antibody:	ABIN2690503
Secondary Antibody:	HRP conjugated donkey anti rabbit antibody (Merck Millipore, AP182P, lot 3043559)
Protocol:	<ul style="list-style-type: none"> <li>Grow Sut2 (xCT<sup>-/-</sup>), MASS20 (xCT<sup>+/+</sup>) and MelanA (xCT<sup>+/+</sup>) cells in RPMI medium (Sigma Aldrich, R8758, lot RNBG5596) supplemented with 10% fetal bovine serum (SAFC, 12303C, lot 16C364) and antibiotics (Penicillin-Streptomycin, Sigma Aldrich, 055M4784V, lot 097M4767V) at 37°C and 5% CO<sub>2</sub> in 60mm tissue culture plates to 80% confluency. Supplement Sut2 cell lines 1M 2-Mercaptoethanol (Sigma Aldrich, SHBF4223V) and TPA (Sigma Aldrich, P1585) 0.1mg/ml. Supplement MelanA cells with TPA.</li> <li>Using 1x Laemmli buffer (4x Laemmli Sample Buffer (Bio-Rad, 161-0747, lot 64098129) containing 20%/vol 2-Mercaptoethanol (Sigma Aldrich, SHBF4223V), diluted 1:4 in PBS).</li> <li>Collect protein lysates using a cell scraper.</li> <li>Heat lysates in a glycerol block for 10min at 99°C. Centrifuge lysates at 14000rpm for 10min at 4°C. Collect supernatant in a 1.5ml microcentrifuge tube and store at -80°C.</li> <li>Thaw samples on ice and transfer 20µl of each lysate to a 0.5ml microcentrifuge tube.</li> <li>Heat lysates in a glycerol block for 5min at 95°C and place them on ice immediately.</li> <li>Separate samples on a freshly cast denaturing 10% SDS-PAGE in a Bio-Rad Mini PROTEAN 3 cell (serial number 525BR 047264) for 2h at 105V alongside protein ladder (Bio-Rad, 61-0374).</li> <li>Transfer proteins in transfer buffer (made in house) onto nitrocellulose membrane (GVS Life Sciences, 1215471, lot number 7051342) in another Bio-Rad Mini PROTEAM 3 cell (serial number 525BR 047264) for 2h at 4°C at 160mA.</li> <li>Confirm successful protein transfer using Ponceau red.</li> </ul>

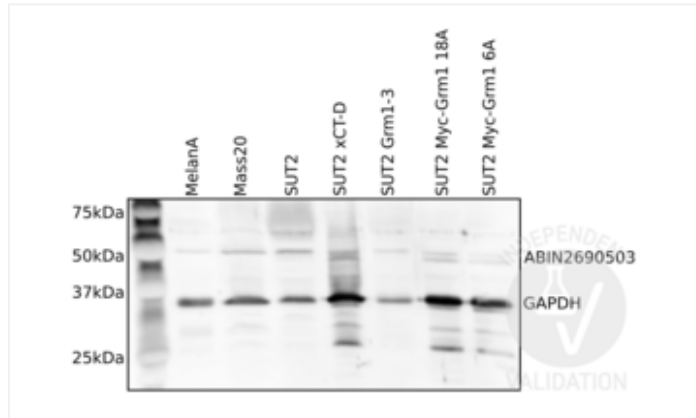
## Validation report #103349 for Western Blotting (WB)

- Block protein using 100ml blocking buffer (transfer buffer containing 0.25% milk) in a Snap i.d. 2.0 (Merck Millipore, C73105).
- Incubate with primary rabbit anti-SLC1A3 antibody (antibodies-online, ABIN2690503, lot 250113/3) diluted 1:10000 and rabbit anti-GAPDH antibody (Santa Cruz Biotechnology, 25778) diluted 1:200 in blocking buffer ON at 4°C.
- Wash membrane 5x in a Snap i.d. 2.0 with 1x TBST.
- Incubation with secondary HRP conjugated donkey anti rabbit antibody (Merck Millipore, AP182P, lot 3043559) diluted 1:5000 in 0.25% milk (powdered goat milk rehydrated in 1X TBST) for 1h at RT.
- Wash membrane 5x in a Snap i.d. 2.0 with 1x TBST.
- Incubate membrane with 2ml Luminata Forte Western HRP Substrate (Merck Millipore, WBLUF0100, 180333) for 3min at RT. Reveal protein bands on a Syngene Gel Documentation system at 5s, 15s, and 30second exposures.

### Experimental Notes:

- ABIN2690503 reveals a band of the expected 60kDa MW in each of the three tested melanocyte extracts. Cell lines transfected with both xCT and Myc-Grm1 appear to have a double band at 60kDa.
- SLC1A3 did not appear to be upregulated in the xCT-/- cell line SUT2.

## Image for Validation report #103349



### Validation image no. 1 for anti-Solute Carrier Family 1 (Glial High Affinity Glutamate Transporter), Member 3 (SLC1A3) (Cytoplasmic Domain) antibody (ABIN2690503)

Western blot analysis with ABIN2690503 of Sut2 (SUT2), MASS20 (Mass20) and MelanA (MelanA) cells extracts and extracts from Sut2 cells transfected with xCT and/or Myc-Grm1. Image exposure 5s.