ION BIOSCIENCES

Instructions

HEK293T GlyT1 (SLC6A9) Recombinant Cell Line

Catalog No. C1102

Introduction

Sodium-dependent glycine transporter 1 (GlyT1, encoded by SLC6A9) is a transmembrane transporter responsible for the reuptake of glycine, the major inhibitory neurotransmitter in the central nervous system. GlyT1 is a member of the SLC6 family of sodium- and chloride-dependent neurotransmitter transporters and operates by coupling glycine transport with the co-transport of sodium and chloride ions across the plasma membrane. The transporter plays a key role in regulating extracellular glycine concentrations, particularly in glycinergic synapses and in modulating N-methyl-D-aspartate (NMDA) receptor activity through glycine clearance.

The human genome encodes two primary glycine transporters—GlyT1 (SLC6A9) and GlyT2 (SLC6A5)—with distinct expression patterns and physiological functions. GlyT1 is predominantly expressed in glial cells throughout the brain and spinal cord and is essential for maintaining glycine homeostasis in excitatory synapses. Dysregulation of GlyT1 function has been implicated in various neurological and psychiatric disorders, including schizophrenia, epilepsy, and cognitive dysfunction. As such, GlyT1 is a promising pharmacological target for the development of novel therapeutics aimed at modulating glycinergic and glutamatergic neurotransmission.

ION Biosciences' HEK293T GlyT1 (SLC6A9) recombinant cell line stably expresses the human glycine transporter 1 and is functionally validated using glycine uptake assays. This model is ideally suited for identifying and profiling small molecule modulators of GlyT1 activity in both high throughput screening and detailed pharmacological studies.

Materials Provided

One vial of HEK293T GlyT1 (SLC6A9) recombinant cells, 2 x 10^6 cells in 1 mL of Bambanker serum free freezing medium.

Storage

Cells are shipped on dry ice and should arrive frozen. To ensure maximum cell viability, store the cell vial in liquid nitrogen immediately upon receipt. (Liquid nitrogen vapor phase only - vials are not rated for liquid immersion).

Mycoplasma testing

The cell line has been screened using ATCC PCR-based testing service which covers 60 species of *Mycoplasma, Acholeplasma, Spiroplasma* and *Ureaplasma* including the eight species most likely to afflict cell cultures: *M. arginini, M. fermentans, M. hominis, M.hyorhinis, M. orale, M. pirum, M. salivarium,* and *A. laidlawii.* The absence of Mycoplasma species has been confirmed for each lot.

Materials Required But Not Supplied

Category	Name	Recommendations
Reagents	Cell culture media	Gibco™ 1X Advanced MEM (ThermoFisher Cat#: 12492013)
	L-glutamine	Gibco™ 100X GlutaMAX™ Supplement (ThermoFisher Cat#: 35050061)
	Fetal bovine serum (FBS)	Corning™ Premium Fetal Bovine Serum (FisherSci Cat#: MT35016CV)
	Puromycin	Gibco™ Puromycin Dihydrochloride (ThermoFisher Cat#: A1113803)
	Trypsin-EDTA	Gibco™ 1X TrypLE™ Express Enzyme (ThermoFisher Cat#: 12605028)
	Phosphate buffered saline	Gibco™ 1X PBS, pH 7.4 (ThermoFisher Cat#: 10010023)
	Freezing media	Bambanker® Serum-Free Cell Freezing Medium (FisherSci Cat#: NC2960954)
Equipment	Single and multichannel micropipettes and pipette tips	
	50 mL and 15 mL conical centrifuge tubes	
	1.5 mL microtubes	
	Tissue culture disposable pipettes (1 mL-25 mL) and tissue culture flasks (e.g. T75 flask)	
	0.2 µm filter unit(s) for medium sterilization	
	Cryovials, cryo-labels, and –1°C/minute Freezing Container for freezing cells	
	Automated Cell Counter (or Hemocytometer)	
	Humidified tissue culture incubator (37°C and 5% CO_2)	

Cell Culture Protocol

A. Cell Culture Media

1. Make **Thawing Medium** by combining 25 mL of FBS, 5 mL of 100X GlutaMax™ with 470 mL 1X Advance MEM media for a final concentration of 5% FBS and 1X GlutaMax™.
2. To make **Complete Cell Culture Medium**, add 150 µL of 10 mg/mL Puromycin into 500 mL of Thawing Medium from **step 1** (or 15 µL of Puromycin for every 50 mL of Thawing Medium) for a final concentration of 3 µg/mL Puromycin.
3. (Optional) Sterilize all media using 0.2 µm filter.
4. Pre-warm all media in a 37°C water bath prior to use.

B. Thawing Cells

1. Aliquot 8 mL of **Thawing Medium** in a 15 mL conical centrifuge tube
2. Place the cryovial in a 37°C water bath briefly, until only small ice crystals remain and the cell pellet is almost completely thawed. The thawing time typically ranges from 2 to 3 minutes. Do **NOT** vortex freshly thawed cells.
3. Spray and wipe the external surface of the cryovial with 70% ethanol. Transfer the vial to a biosafety cabinet.
4. Gently add the cells into the pre-filled 15 mL conical tube dropwise.

Cell Culture Protocol (Continued)

5. Rinse the cryovial with 1 mL of **Thawing Medium** to maximize cell recovery and add it back into the 15 mL conical tube.
6. Centrifuge the 15 mL conical tube containing the cells at 500 x g for 5 min at 25°C.
7. After centrifugation, carefully aspirate the supernatant without disturbing the cell pellet.
8. Gently resuspend the cell pellet with 1 mL of **Complete Cell Culture Medium** .
9. Add 14 mL of **Complete Cell Culture Medium** into a T75 flask.
10. Transfer the cell suspension to the T75 flask and gently swirl the flask to distribute the cells evenly in the solution.
11. Incubate the flask in a 37°C and 5% CO_2 humidified cell culture incubator.
12. Maintain the cells in culture by changing culture medium every 2-3 days, until they reach >80% confluency in a T75.

C. Cell Passage

1. Remove the T75 flask from the tissue culture incubator and place it in a biosafety cabinet.
2. Gently aspirate the media from the T75 flask.
3. Add 15 mL of PBS into the T75 flask, and gently rock the flask back and forth to rinse the cells.
4. Gently aspirate PBS from the flask.
5. Add 3 mL of pre-warmed TrypLE™ to the flask. Gently rock the flask back and forth to ensure that the flask is uniformly covered with TrypLE™.
6. Incubate the flask at 37°C and 5% CO_2 until the cells have detached. Usually it will take 8 - 10 min.
7. Remove the flask from the incubator and observe the cells under a microscope to confirm cell detachment. If necessary, gently tap the edge of the flask to detach cells from the surface.
8. Add 10 mL of pre-warmed **Complete Cell Culture Medium** to the detached cells in the flask.
9. Transfer cell suspension into a 15 mL conical tube.
10. Centrifuge the 15 mL conical tube containing cells at 500 x g for 5 min at 25°C.
11. After centrifugation, carefully aspirate the supernatant without disturbing the cell pellet.
12. Resuspend the cell pellet with 5 mL of **Complete Cell Culture Medium**. Pipette the cell suspension up and down several times to generate a single cell suspension without any clumps.
13. Take a small aliquot of the cell suspension (~20 µL) for cell counting, and determine the number of cells and volume required to seed into a T75 flask. HEK293-T GlyT1 (SLC6A9) cells have a doubling time of ~24 hours. A confluent T75 flask normally yields ~10 x 10^6 cells. We recommend to seed 1 x 10^6 cells for a T75 flask in three to four days.
14. Add 15 mL of **Complete Cell Culture Medium** into a new T75 flask, followed by addition of the appropriate volume of cell suspension. Transfer the flask to a tissue culture incubator, and incubate the cells at 37°C and 5%CO_2.

Cell Culture Protocol (Continued)

D. Cryopreservation

1. Harvest cells according to the description in **Section C Cell Passage steps 1 - 9**.
2. Set aside a small fraction of the suspended cells (~20 µL) in a separate tube for cell counting.
3. Count cells, calculate the concentration of cells and the total number of cells in the original 15 mL conical tube.
4. Centrifuge the 15 mL conical tube containing cells at 500 x g for 5 min at 25°C.
5. After centrifugation, discard the supernatant, being careful not to disturb the cell pellet.
6. Based on the total cell number calculated in **Step 3**, resuspend the cells to the desired concentration (e.g. 2.0 x 10^6 cells/mL) in Bambanker® Serum-Free Cell Freezing Medium.
7. Aliquot 1 mL of the cell suspension into labeled 2 mL cryovials. Seal the cryovials tightly.
8. Freeze cells in a -80°C freezer at a controlled rate of -1°C/minute overnight in a cell freezing container.
9. The following day, transfer the vials into the vapor phase of liquid nitrogen for long-term storage.

Functional Validation

The biological function of the HEK293T GlyT1 (SLC6A9) recombinant cell line was validated using glycine, the primary substrate of GlyT1. As the brain's principal inhibitory neurotransmitter, glycine is regulated by GlyT1 through synaptic reuptake. This transport activity has a reported EC_{50} of 23 µM. Using ION's Sodium-Dependent SLC Transporters Assay Kit, cells were pre-incubated with the sodium-sensitive fluorescent dye ING-2 AM for 1 hour, followed by treatment with increasing concentrations of glycine. Transport of glycine via GlyT1 facilitates sodium co-transport into the cells, where sodium binds to ING-2 and generates a fluorescent signal. Fluorescence was recorded for 30 seconds to establish a baseline, then continuously monitored for an additional 270 seconds following glycine addition (Figure 1). V_{max} was calculated as the slope during the first 30 seconds post-stimulation, and AUC was derived from the area under the response curve relative to baseline. Dose–response curves plotted glycine concentration against either V_{max} or AUC, yielding an EC_{50} of 7.3 µM, which is consistent with the reported value.

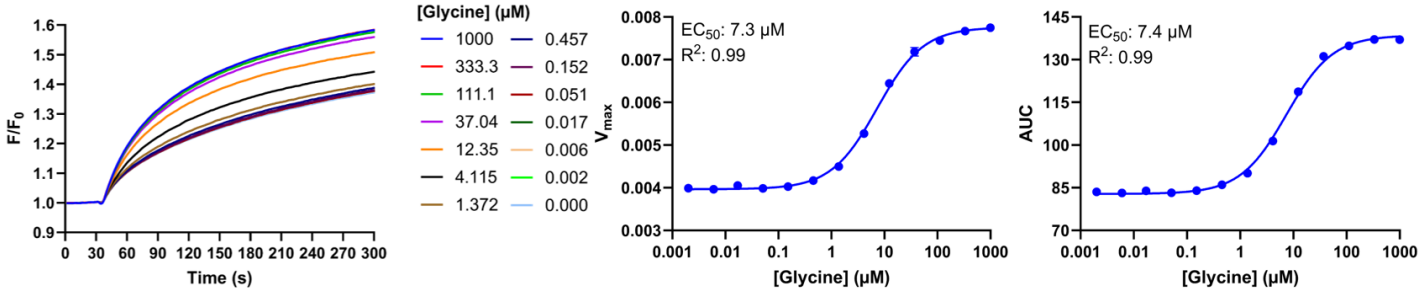

Figure 1. Glycine uptake in HEK293T GlyT1 (SLC6A9) Recombinant Cell Line. Baseline-normalized kinetic fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). Glycine, the primary substrate for the GlyT1 transporter, was added at 30 seconds,. The V_{max} was calculated from the first 30 seconds following stimulus addition. The AUC was calculated between the curve a y=1. Error bars indicate the SEM (n=3). The EC_{50} for glycine uptake is about 7 µM.

Functional Validation (Continued)

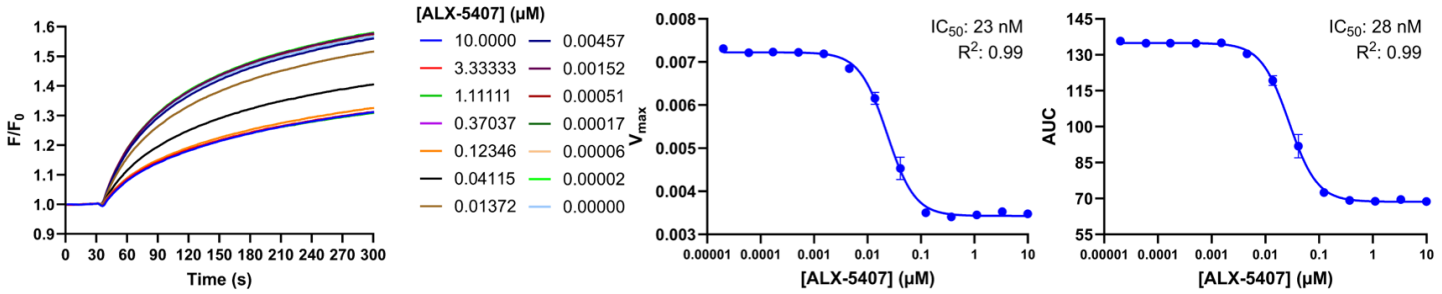

Figure 2. ALX-5407 Inhibition of Glycine Uptake in HEK293T GlyT1 (SLC6A9) Recombinant Cell Line. Baseline-normalized kinetic fluorescence data obtained using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). ALX-5407, an inhibitor of the GlyT1 transporter, was pre-incubated for 15 minutes before the start of the assay. Glycine, the primary substrate for the GlyT1 transporter, was added at 30 seconds at a concentration of 1 mM. The V_{max} was calculated from the first 30 seconds following stimulus addition. The AUC was calculated between the curve and y=1. Error bars indicate the SEM (n=3). The IC_{50} for ALX-5407 inhibition of glycine uptake is about 23 nM.

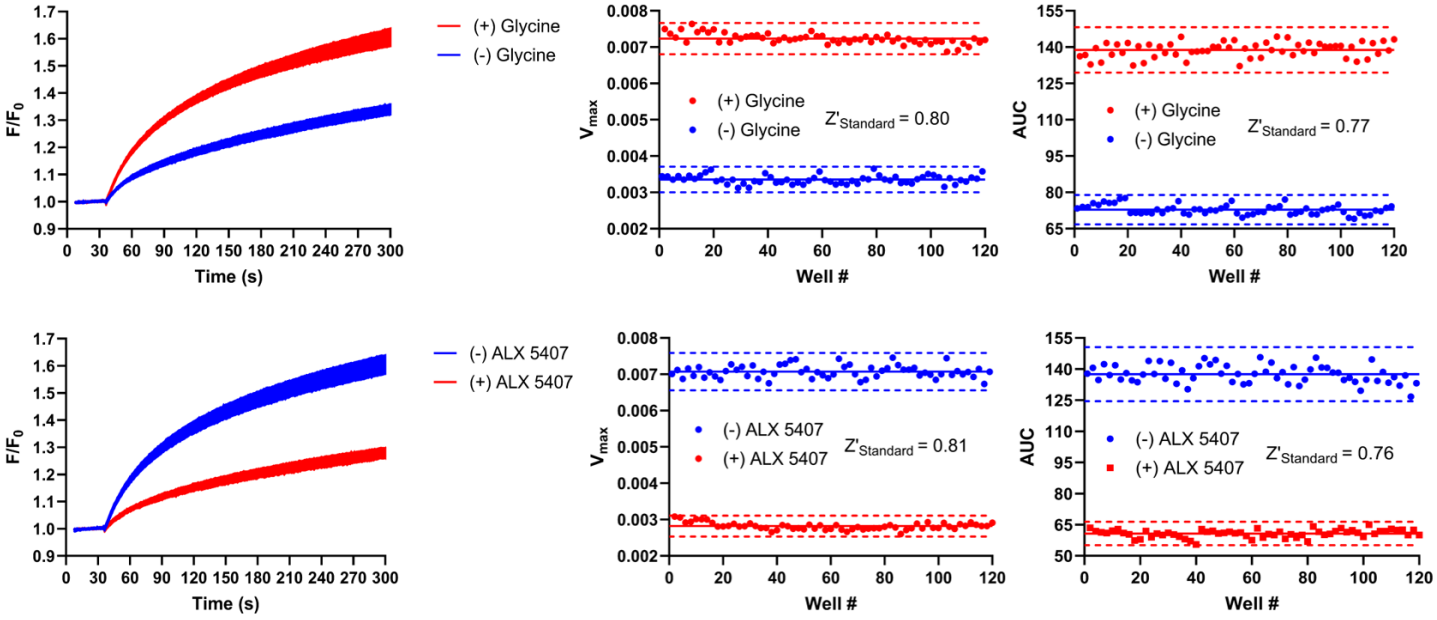

Figure 3. Z' Determination of HEK293T GlyT1 (SLC6A9) Recombinant Cell Line. Baseline-normalized kinetic fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). ±ALX-5407, an inhibitor of the GlyT1 transporter, was pre-incubated for 15 minutes at a concentration of 10 µM before the start of the assay. ±Glycine, the primary substrate for the GlyT1 transporter, was added at 30 seconds at a concentration of 1 mM. The V_{max} was calculated from the first 30 seconds following stimulus addition. The AUC was calculated between the curve and y=1. The Z' factor plots show a Z' above 0.7 for both glycine uptake and ALX-5407 inhibition of glycine uptake (n = 60).

Vector and Sequence

Vector description

Mammalian Gene Expression Lentiviral Vector, pLV[Exp]-CMV>hSLC6A9[NM_201649.4]:IRES:Puro

hSLC6A9 sequence [NM_201649.4]

MSGGDTRAAIARPRMAAAHGPVAPSSPEQVTLLPVQRSFFLPPFSGATPSTSLAESVLKVWHGAYNSGLLPQLMAQHSLAMAQNGAVPSEATKRD
QNLKRGNWGNQIEFVLTSVGYAVGLGNVWRFPYLCYRNGGGAFMFPYFIMLIFCGIPLFFMELSFGQFASQGCLGVWRISPMFKGVGYGMMVVSTYI
GIYYNVVICIAFYYFFSSMTHVLPWAYCNNPWNTHDCAGVLDASNLTNGSRPAALPSNLSHLLNHSLQRTSPSEEYWRLYVLKLSDDIGNFGEVRLPL
LGCLGVSWLVVFLCLIRGVKSSGKVVYFTATFPYVVLTILFVRGVTLEGAFDGIMYYLTPQWDKILEAKVWGDAASQIFYSLGCAWGGLITMASYNKFH
NNCYRDSVIISITNCATSVYAGFVIFSILGFMANHLGVDVSRVADHGPGLAFVAYPEALTLLPISPLWSLLFFFMLILLGLGTQFCLLETLVTAIVDEVGN
EWILQKKTYVTLGVAVAGFLLGIPLTSQAGIYWLLLMDNYAASFSLVVISCIMCVAIMYIYGHRNYFQDIQMMLGFPPPLFFQICWRFVSPAIIFFILVFT
VIQYQPITYNHYQYPGWAVAIGFLMALSSVLCIPLYAMFRLCRTDGDTLLQRLKNATKPSRDWGPALLEHRTGRYAPTIAPSPEDGFEVQPLHPDKAQI
PIVGSNGSSRLQDSRI

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