ION BIOSCIENCES

Instructions

HEK293T GAT1 (SLC6A1) Cell Line

Catalog No. C1104

Introduction

The GABA transporter 1 (GAT1), encoded by the SLC6A1 gene, is a sodium- and chloride-dependent symporter responsible for the high-affinity reuptake of γ-aminobutyric acid (GABA) from the synaptic cleft into presynaptic neurons and surrounding glial cells. By regulating extracellular GABA concentrations, GAT1 plays a key role in maintaining inhibitory tone and synaptic homeostasis throughout the central nervous system, particularly in regions such as the hippocampus, cortex, and cerebellum. Dysregulation of GAT1 function has been implicated in a variety of neurological and psychiatric disorders, including epilepsy, autism spectrum disorder, anxiety, and schizophrenia.

SLC6A1 is a member of the SLC6 family of neurotransmitter transporters and shares the conserved 12-transmembrane domain topology characteristic of this family. GAT1 couples the transport of GABA with the inward movement of sodium and chloride ions, leveraging electrochemical gradients to drive uptake against concentration gradients. This mechanism is essential for the rapid clearance of GABA from synapses and for shaping inhibitory signaling dynamics.

ION Biosciences' HEK293T GAT1 (SLC6A1) recombinant cell line stably expresses the human GABA transporter and provides a validated in vitro model for studying GAT1 function and pharmacology. This cell line is optimized for high-throughput screening of GAT1 inhibitors or substrates, functional uptake assays, and kinetic studies of transporter activity. Its strong, reproducible, sodium-dependent GABA uptake and compatibility with fluorescence-based assays make it an ideal platform for advancing drug discovery efforts targeting GABAergic pathways in CNS disease.

Materials Provided

One vial of HEK293T GAT1 (SLC6A1) 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[1]	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)
	Dissociation Reagent	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)	

[1] *If desired, antibiotics such as Penicillin and Streptomycin may be supplemented to prevent bacterial infection. We recommend using Gibco™ Penicillin Streptomycin GlutaMAX™ Supplement (Thermo Fisher Cat#: A5873601) in place of GlutaMAX™.*

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.

Cell Culture Protocol (Continued)

4. Gently add the cells into the pre-filled 15 mL conical tube dropwise.
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 GAT1 (SLC6A1) 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 GAT1 (SLC6A1) recombinant cell line was validated using γ-aminobutyric acid (GABA), the primary substrate for GAT1, and NNC-711, a selective GAT1 inhibitor. Functional activity was assessed using ION Biosciences' Sodium-Dependent SLC Transporter Assay Kit, which employs the sodium-sensitive fluorescent dye ING-2 to detect transporter-mediated sodium influx. Cells were pre-loaded with ING-2 and incubated for 1 hour, followed by treatment with either increasing concentrations of GABA or a fixed concentration of GABA combined with increasing concentrations of NNC-711. GABA uptake via GAT1 is coupled with sodium co-transport, leading to a rapid increase in intracellular sodium and a corresponding rise in fluorescence. NNC-711 produced a dose-dependent inhibition of this signal, confirming specific blockade of GAT1 function. Kinetic responses were quantified by calculating the initial rate of fluorescence change (V_{max}) and the area under the fluorescence curve (AUC) following stimulation. The observed EC_{50} for GABA and IC_{50} for NNC-711 were consistent with literature values, confirming the functional expression and pharmacological relevance of the HEK293T GAT1 (SLC6A1) recombinant cell line.

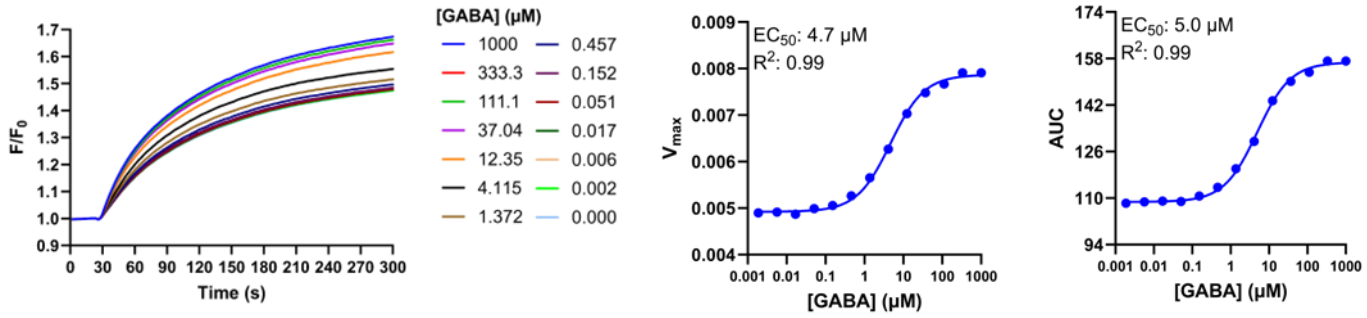

Figure 1. GABA dose response on HEK293T GAT1 (SLC6A1) recombinant cell line using ION Biosciences' Sodium-dependent SLC Transporter Assay Kit[2]. Baseline-normalized fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562 (40) nm). GABA, the primary substrate for GAT1, 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 and y=1. Error bars indicate the SEM (n = 3). The EC_{50} for GABA uptake is about 5 µM.

Functional Validation (Continued)

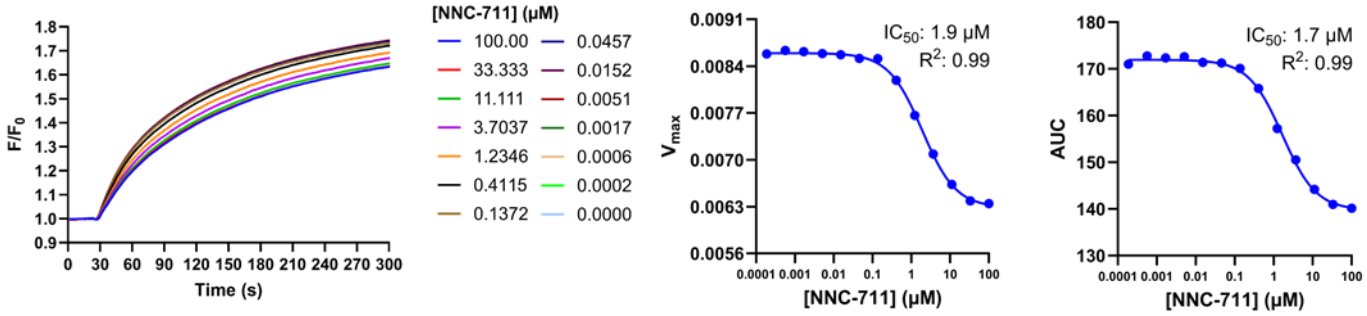

Figure 2. NNC-711 dose response on HEK293T GAT1 (SLC6A1) recombinant cell line using ION Biosciences' Sodium-dependent SLC Transporter Assay Kit[2]. Baseline-normalized fluorescence data collected using Wavefront Panoptic (Excitation: 518 nm, Emission: 562(40) nm). NNC-711, a selective GAT1 inhibitor, was pre-incubated for 15 minutes before the start of the assay. GABA, the primary substrate for GAT1, 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 NNC-711 inhibition of GAT1 is about 2 µM.

[2] Compatible Assay Kits

ION Biosciences has Assay Kits available that provide all the reagents needed for testing your GABA substrates and GAT1 modulators for 2 or 10 plates in both 96 and 384 well plate formats, and are compatible with our HEK293T GAT1 (SLC6A1) cell line. Assay kits are available for direct purchase from our website or by contacting our Sales Department.

Name	Sizes	Catalog #
Sodium-dependent SLC Transporter Assay Kit	pIONeer - 2 Plates Flex - 10 Plates	8000-2 8000-10

Vector and Sequence

Vector description

Mammalian Gene Expression Vector, pRP[Exp]-Puro-CAG>hSLC6A1[NM_003042.4]

hSLC6A1 Sequence [NM_003042.4]

MATNGSKVADGQISTEVSEAPVANDKPKTLVVKVQKKAADLPDRDTWKGRFDFLMSCVGYAIGLGNVWRFPYLCGKNGGGAFLIPYFL
TLIFAGVPLFLLECSLGQYTSIGGLGVWKLAPMFKGVGLAAAVLSFWLNIYYIVIISWAIYYLYNSFTTTLPWKQCDNPWNTDRCFSNYS
MVNTTNMTSAVVEFWERNMHQMTDGLDKPGQIRWPLAITLAIAWILVYFCIWKGVGWTGKVVYFSATYPYIMLIILFFRGVTLPGAKEG
ILFYITPNFRKLSDSEVWLDAATQIFFSYGLGLGSLIALGSYNSFHNNVYRDSIIVCCINSCTSMFAGFVIFSIVGFMAHVTKRSIADVAASG
PGLAFLAYPEAVTQLPISPLWAILFFSMLLMLGIDSQFCTVEGFITALVDEYPRLLRNRRELFIAAVCIISYLIGLSNITQGGIYVFKLFDYYS
ASGMSLLFLVFFECVSISWFYGVNRFYDNIQEMVGSRPCIWWKLCWSFFTPIIVAGVFIFSAVQMTPLTMGNYVFPKWGQGVGWLMAL
SSMVLIPGYMAYMFLTLKGSLKQRIQVMVQPSEDIVRPENGPEQPQAGSSTSKEAYI

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