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Data Sheet

SLC5A5 – HEK293 Recombinant Cell line

Catalog #:90333

Description

Stable recombinant HEK293 cell line expressing human SLC5A5 (UniProt # Q92911) with a C-terminal FLAG-tag. SLC5A5 is a sodium-dependent iodide transporter and is a member of the sodium glucose co-transporter family. The expression of SLC5A5 is confirmed by Western blotting. This cell line can be used to evaluate agents that may affect iodide transport.

Background

Iodine transport is essential for the production of thyroid hormone (T4). Sodium Iodide Symporter SLC5A5 (NIS) is a sodium-dependent transporter of iodide into thyroid follicular cells and other peripheral tissues to support T4 synthesis [1]. Lack of iodide transport can result in hypothyroidism and can manifest itself in several disease states like goiter formation and weight gain. SLC5A5 also plays an important role in hyperthyroidism (an excessive production of T4). Expression and activity of SCL5A5 is altered in several forms of cancer. Inhibitors of SLC5A5 have potential therapeutic benefit for the regulation of iodine overload induced by amiodarone or other thyromimetics that can result in hyperthyroidism [2].

Applications

- Drug compound screening
- Functional assays

Format

Each vial contains 1.5 X 10^6 cells in 1 ml of 10% DMSO

Host cell

HEK293 cells

Recommended Storage

Immediately upon receipt, store in liquid nitrogen.

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Functional Validation and Assay Performance

The following assays are designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.

Culture Conditions

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01)

Growth Medium 1B (BPS Cat. #79531): Thaw Medium 1 (BPS Cat. #60187) plus 400 µg/ml of Geneticin (Life Technologies #11811031).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1B.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 **(no Geneticin)**, spin down cells, and resuspend cells in pre-warmed Thaw Medium 1 **(no Geneticin)**. Transfer resuspended cells to a T25 flask and culture in a CO_2 incubator at 37°C. At first passage, switch to Growth Medium 1B. Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel using 0.05% Trypsin/EDTA, and add Growth Medium 1B. Transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20 weekly.

Materials Required but Not Supplied

- Assay Medium: Opti-MEM I (Life technologies #31985-062), 0.5% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, and 1% Pen/Strep
- 96-well tissue culture plate coated with poly-D-lysine
  [We used 0.1 mg/ml poly-D-lysine (Sigma #P6407) in H_2O for 1 hour at RT.]
- Hanks Buffered Salt Solution (HBSS, Hyclone #SH30588.01)
- Reagents for the Ce/As reaction to determine iodide concentration as reported previously [3]
  - 4x Ammonium cerium (IV) sulfate solution (4x Ce, 42 mM)
  - 4x Sodium arsenite solution (4x As, 96 mM)
- Iodide standards (0- 1 µM NaI, Sigma #383112)
- Plate reader for measuring absorbance at 420 nm

Transport activity of – HEK293 stably expressing SLC5A5

1. Harvest SLC5A5 – HEK293 cells from culture in Growth Medium 1B and seed cells at a density of ~ 25,000 cells per well into a white clear-bottom 96-well microplate in

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100 µl of Assay Medium. Make sure to leave two columns of the plate (16 wells total) empty for the iodide standards.

2. Incubate cells at 37°C with 5% CO_2 overnight.

3. The next day, carefully remove the medium from the wells. Wash cells once with Hanks Buffered Salt Solution (HBSS, Hyclone #SH30588.01) and then add 80 µl HBSS to each well.

4. Pretreat cells with vehicle, ITB5 (Chembridge #5461570) or any test inhibitors by adding 10 µl of 10x stock solutions to each well and incubating for 30 minutes at RT. Ideally, it is best to keep the final DMSO concentrations at 0.1% or less.

5. Begin the uptake assay by adding 10 µl of 100 µM NaI (Sigma #383112) diluted in HBSS (final NaI concentration of 10 µM) and incubate for 1h at RT.

6. Uptake should them be terminated by carefully washing twice with 200 µl cold HBSS, making sure not to disrupt the cell layer.

7. Accumulated iodide can be measured using the Ce/As method as previously described [3].

   Protocol for Ce/As Method
   - Dilute 4x Ce and 4x As solutions to 1x with H_2O immediately before reaction
   - After the final wash with HBSS (step 6), add 100 µl H_2O to wells.
     - Prepare and add 100 µl iodide standards (S0- S7) to empty columns in duplicate (0, 100, 250, 400, 550, 700, 850, 1000 nM respectively).
   - Add 100 µl 1x Ce solution to each well
   - Add 100 µl 1x As solution to each well
   - Allow reaction to continue for 20 min at RT
   - Measure absorbance at 420 nm (A420)

8. Data Analysis: log [A_{420}] for samples was normalized to the iodide standards to determine pmol iodide accumulated per well. Data was analyzed with GraphPad Prism.

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Sample Data

Figure 1. Concentration dependent uptake of Iodide by SLC5A5 - HEK293 cells. WT or SLC5A5 – HEK293 cells were incubated with the indicated concentrations of NaI (0-100 µM) for 1h at RT. Iodide uptake was measured using the Ce/As protocol described above and normalized to freshly made iodide standards.

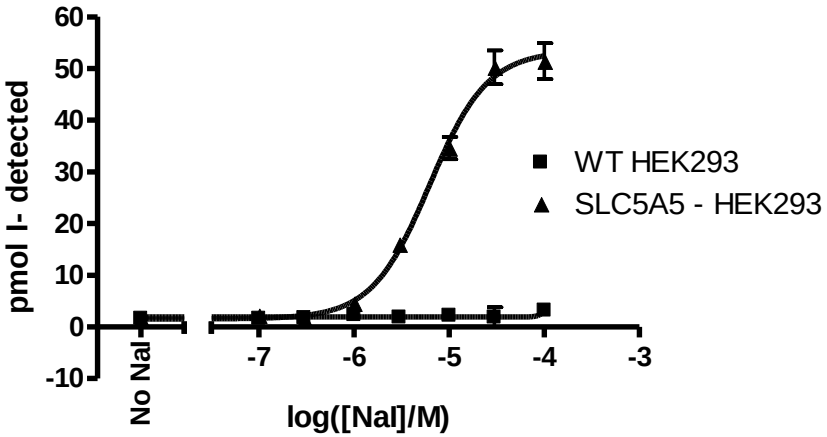

Figure 2. Time dependent uptake of Iodide by SLC5A5 - HEK293 cells. WT or SLC5A5 – HEK293 cells were incubated with 5 µM NaI for the indicated times (0-240 min) at RT. Additions of NaI were staggered to allow termination of the uptake reaction at the 240 min. timepoint. Iodide uptake was measured using the Ce/As protocol described above and normalized to freshly made iodide standards.

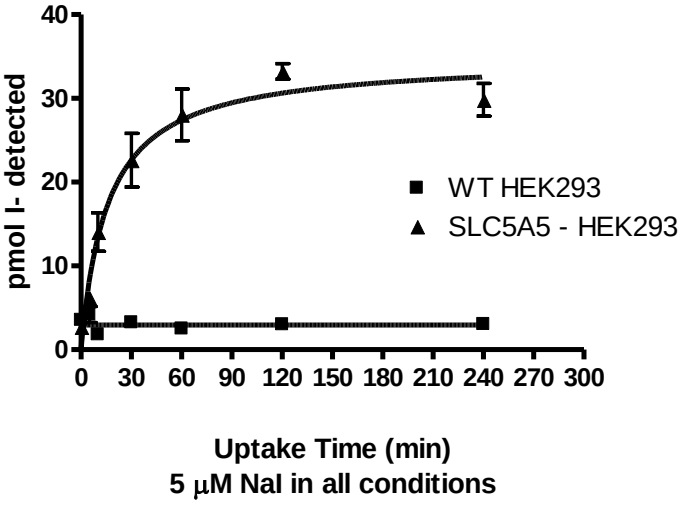

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Figure 3. ITB5 dependent inhibition of Iodide uptake by SLC5A5 - HEK293 cells. WT or SLC5A5 – HEK293 cells were pre with the indicated concentrations of the known SLC5A5 inhibitor ITB5 (0-30 µM, Chembridge # 5461570) for 30 minutes at RT. Subsequently, 5 µM NaI (final concentration) was added to the reaction and incubated for 1h at RT. Iodide uptake was measured using the Ce/As protocol described above and normalized to freshly made iodide standards.

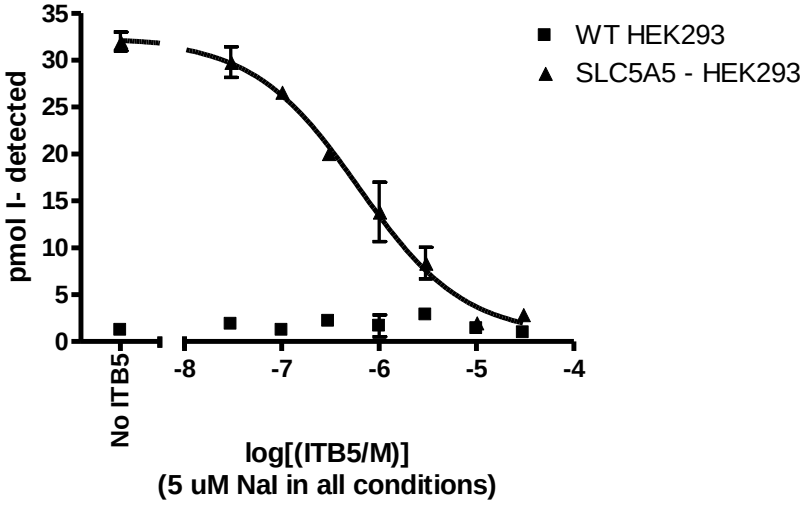
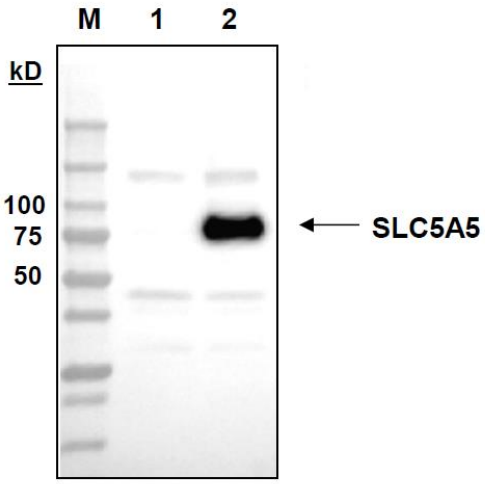


Figure 4. Western Blot of the SLC5A5 expressing cells. Western Blot of WT HEK293 cells (lane 1) and SLC5A5 - HEK293 cells (lane 2) stained with rabbit anti-FLAG (Sigma # F7425) M: molecular weight marker.



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Vector and Sequence

MEAVETGERPTFGAWDYGVFALMLLVSTGIGLWVGLARGGQRSAEDFFTGGRRLAALPVGLS
LSASFMSAVQVLGVPSEAYRYGLKFLWMCLGQLLNSVLTALLFMPVFYRLGLTSTYEYLEMRF
SRAVRLCGTLQYIVATMLYTGIVIYAPALILNQVTGLDIWASLLSTGIICTFYTAVGGMKAVVWTD
VFQVVVMLSGFWVVLARGVMLVGGPRQVLTLAQNHSRINLMDFNPDPRSRYTFWTFVVGGTL
VWLSMYGVNQAQVQRYVACRTEKQAKLALLINQVGLFLIVSSAACCGIVMFVFYTDCDPLLLGR
ISAPDQYMPLLVLDIFEDLPGVPGLFLACAYSGTLSTASTSINAMAAVTVEDLIKPRLRSLAPRKL
VIISKGLSLIYGSACLTVAALSSLLGGGVLQGSFTVMGVISGPLLGAFILGMFLPACNTPGVLAGL
GAGLALSLWVALGATLYPPSEQTMRVLPSSAARCVALSVNASGLLDPALLPANDSSRAPSSGM
DASRPALADSFYAISYLYYGALGTLTTVLCGALISCLTGPTKRSTLAPGLLWWDLARQTASVAP
KEEVAILDDNLVKGPEELPTGNKKPPGFLPTNEDRLFFLGQKELEGAGSWTPCVGHDGGRDQ
QETNLAADYKDDDDK

Stability

The cell line has been demonstrated to be stable for at least seven continuous passages. For optimal results, it is recommended to use the cells prior to the 7th passage. Upon receipt, amplify the cells in culture and make several frozen aliquots for future use.

References

1. Dohán et al., (2003) The sodium/iodide Symporter (NIS): characterization, regulation, and medical significance. *Endocrine Reviews* **24(1)**:48-77.
2. Lindenthal *et al.*, (2009) Characterization of NIS inhibitors. *Journal of Endocrinology* **200**: 357–365.
3. Waltz *et al.*, (2010) A nonradioactive iodide uptake assay for sodium iodide symporter function. *Analytical Biochemistry* **396(1)**: 91-5.

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