Description

The URAT1 HEK293 Cell Line is an engineered HEK293 cell line that expresses stably integrated human URAT1 (Urate Transporter 1). This cell line is ideal for measuring URAT1-mediated transport using fluorescent substrates.

This cell line has been validated by measuring the URAT1-mediated cellular uptake of 6-FAM (6-carboxyfluorescein), which shares similar anion properties with uric acid, as well as by assessing the inhibition of its uptake by URAT1 inhibitors.

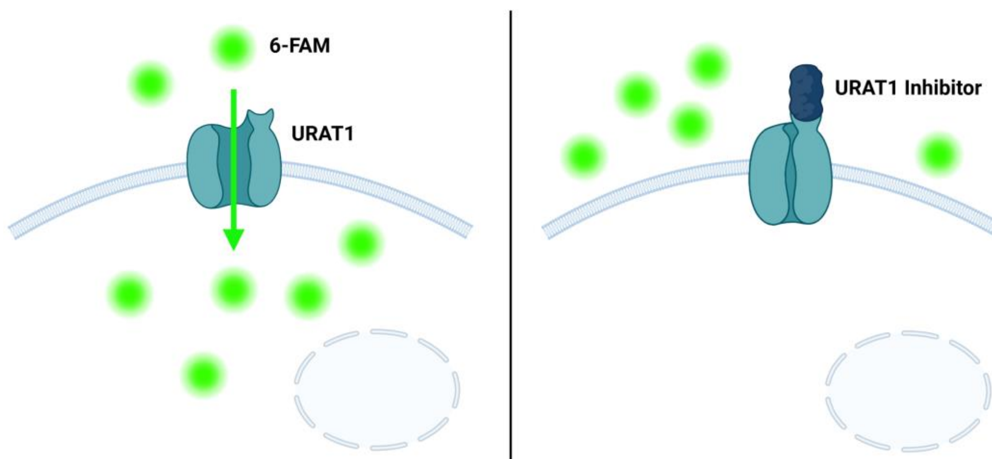

Figure 1. Illustration of the uptake of 6-FAM mediated by URAT1 and the inhibition of this uptake by a URAT1 inhibitor in the URAT1 HEK293 Cell Line.

Background

URAT1 (Urate Transporter 1), encoded by the SLC22A12 gene, is a transmembrane protein localized on the apical membrane of renal proximal tubule cells. Its primary physiological role is to act as a urate-anion exchanger, responsible for reabsorbing approximately 90% of the uric acid filtered by the kidneys back into the bloodstream. Thus, URAT1 is the central gatekeeper of uric acid homeostasis. Gain-of-function mutations lead to hyperuricemia, the precursor to gout, kidney stones, and associated cardiovascular risks. Conversely, loss-of-function mutations result in idiopathic renal hypouricemia, a condition characterized by abnormally low blood urate levels and an increased risk of exercise-induced acute kidney injury. URAT1 inhibitors, or uricosurics, block the transporter and increase uric acid secretion.

Application

- Monitor the cellular transport activity of URAT1 in response to fluorescent substrates.
- Screen URAT1 inhibitors.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

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Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1N	BPS Bioscience #79801

Materials Required for Cellular Assay

Name	Ordering Information
URAT1 Cellular Transport Medium	BPS Bioscience #84225
URAT1 Cellular Transport Lysis Buffer	BPS Bioscience #84224
6-FAM (6-carboxyfluorescein)	BPS Bioscience #84227
Benzbromarone	BPS Bioscience #84228
Verinurad	BPS Bioscience #84229
Epaminurad	BPS Bioscience #84230
Corning BioCoat™ Poly D-Lysine 96-well Black/Clear Flat Bottom plates	Corning #354640
Fluorometer or plate reader capable of reading at 484/530 nm	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

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Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1N (BPS Bioscience #79801):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 0.5 µg/ml of Puromycin.

Cell Culture Protocol
Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1N**.**

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1N and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1N.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:6 to 1:8 twice per week.

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Cell Freezing

1. Aspirate the medium, wash the cells PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 1 and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation

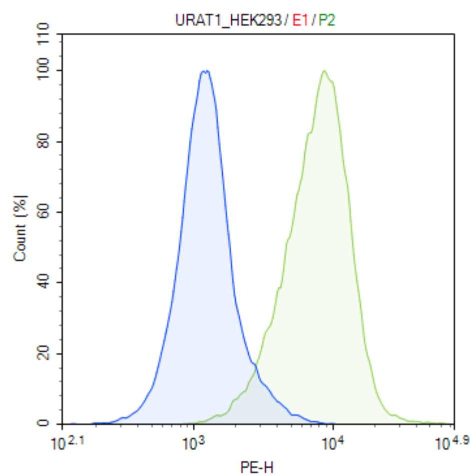

Figure 2. Flow cytometry analysis of URAT1 cell surface expression in the URAT1 HEK293 Cell Line. URAT1 HEK293 cells (green) and parental HEK293 cells (blue) were stained with an anti- SLC22A12 antibody along with PE-labeled goat anti-mouse IgG antibody, and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates PE intensity.

Functional Validation

- 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.
- All conditions should be performed in triplicate.
- Assay A should include “Treated Cells”, “Background Control” and “Untreated Control” conditions.
- Assay B should include “Treated Cells, No Compound”, “Treated Cells, Test Compound”, “Background Control” and “Untreated Control” conditions.
- It is recommended to use a final concentration of DMSO at 0.1% or less for optimal results.

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A. URAT1-Based Cellular Transport Fluorescent Assay

1. Seed URAT1 HEK293 cells at a density of 50,000 cells per well in 100 µl/well of Thaw Medium 1 into a Poly-D-Lysine-coated 96-well black/clear flat bottom cell culture plate. Leave empty wells as cell-free control wells ("Background Control").

2. Incubate the cells at 37°C with 5% CO_2 for 24 hours.

3. Remove the culture medium using a multi-channel pipette: tilt the plate, position tips against the side wall to avoid disturbing cells, and use a slow, steady aspiration.

4. Add 90 µl of URAT1 Cellular Transport Medium and let the plate sit at room temperature (RT).

5. Prepare a serial dilution of 6-FAM at 10-fold the final concentration in Cellular Transport Medium (10 µl/well).

   Note: 6-FAM is light sensitive, avoid exposure to direct light.

6. Add 10 µl of the diluted 6-FAM to the "Treated Cells" wells.

7. Add 10 µl of URAT1 Cellular Transport Medium to the "Untreated Control" wells.

8. Add 100 µl of URAT1 Cellular Transport Medium to the "Background Control" wells (cell-free wells).

9. Incubate the cells at RT for 1 hour in the dark.

10. Remove URAT1 Cellular Transport Medium using a multi-channel pipette as described in step 3.

11. Wash the cells by adding 200 µl of URAT1 Cellular Transport Medium and aspirate as described in step 3 (repeat the wash step 3 times).

12. Add 100 µl of URAT1 Cellular Transport Lysis Buffer to each well.

13. Incubate the plates at RT for ~30 minutes with gentle agitation in the dark.

14. Measure fluorescence at an excitation wavelength of 484 nm and an emission wavelength of 530 nm using a fluorometer.

15. Data Analysis: Subtract the average fluorescence value of the "Background Control" from the fluorescence readings of all other wells.

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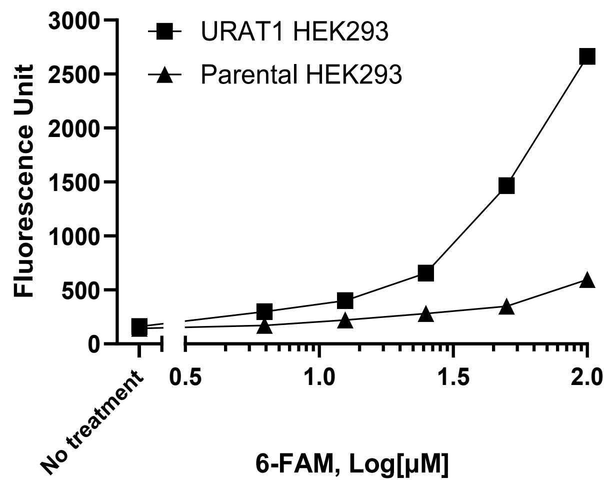

Figure 3. Analysis of URAT1-mediated transport of 6-FAM in the URAT1 HEK293 Cell Line.
URAT1 HEK293 cells were treated with increasing concentrations of 6-FAM for 1 hour and washed extensively. The uptake of 6-FAM was evaluated by measuring fluorescence intensity.

B. URAT1-Based Cellular Transport Inhibition Fluorescent Assay

1. Seed URAT1 HEK293 cells at a density of 50,000 cells per well in 100 µl/well of Thaw Medium 1 into a Poly-D-Lysine-coated 96-well black/clear flat bottom cell culture plate. Leave empty wells as cell-free control wells ("Background Control").

2. Incubate the cells at 37°C with 5% CO_2 for 24 hours.

3. Remove the culture medium using a multi-channel pipette: tilt the plate, position tips against the side wall to avoid disturbing cells, and use a slow, steady aspiration.

4. Add 80 µl of URAT1 Cellular Transport Medium and let the plate sit at RT.

5. Prepare a serial dilution of Benzbromarone at 10-fold the final testing concentration in URAT1 Cellular Transport Medium (10 µl/well).

   Note: For example, to test 100 nM Benzbromarone, prepare 100 µM Benzbromarone in DMSO and dilute it 100-fold in URAT1 Cellular Transport Medium. This results in a 1 µM Benzbromarone solution in URAT1 Cellular Transport Medium containing 1% DMSO. This is the diluted Benzbromarone at 10-fold the final testing concentration. It is recommended to use a final concentration of DMSO at 0.1% or less for optimal results.

6. Add 10 µl of the diluted Benzbromarone to the "Treated Cells, Test Compound" wells.

7. Add 10 µl of URAT1 Cellular Transport Medium containing 1% DMSO to the "Treated Cells, No Compound" and "Untreated Control" (100% transport activity and 0% transport activity, respectively).

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8. Incubate the cells at RT for at least 5 minutes.

9. Prepare 6-FAM in URAT1 Cellular Transport Medium at 10-fold the final concentration, i.e. for a treatment of 6-FAM at 100 µM (10 µl/well).

   Note: 6-FAM is light sensitive, avoid exposure to direct light.

10. Add 10 µl of diluted 6-FAM to the "Treated Cells, Test Compound" and "Treated Cells, No Compound" wells.

11. Add 10 µl of URAT1 Cellular Transport Medium to the "Untreated" wells.

12. Add 100 µl of URAT1 Cellular Transport Medium to the "Background Control" wells.

13. Incubate the cells at RT for 1 hour in the dark.

14. Remove the URAT1 Cellular Transport Medium using a multi-channel pipette as described in step 3.

15. Wash the cells by adding 200 µl of URAT1 Cellular Transport Medium and aspirate as described in step 3 (repeat the wash step 3-5 times).

16. Add 100 µl of URAT1 Cellular Transport Lysis Buffer and incubate the plates at RT for ~30 minutes with gentle agitation in the dark.

17. Measure fluorescence at an excitation wavelength of 484 nm and an emission wavelength of 530 nm using a fluorometer.

18. Data Analysis: Subtract the average fluorescence value of the "Background Control" from the fluorescence readings of all other wells. The percent fluorescence is the average background control-subtracted fluorescence of the "Treated Cells, Test Compound" treated wells divided by the average background control-subtracted fluorescence of the "Treated Cells, No Compound" wells (6-FAM added but no Benzbromarone) multiplied by 100.

$$\%\ Fluorescence = \left(\frac{Fluorescence\ of\ Treated, Test\ Compount - avg.background}{Avg.Fluorescence\ of\ Treated, No\ Compound - avg.background}\right) X\ 100$$

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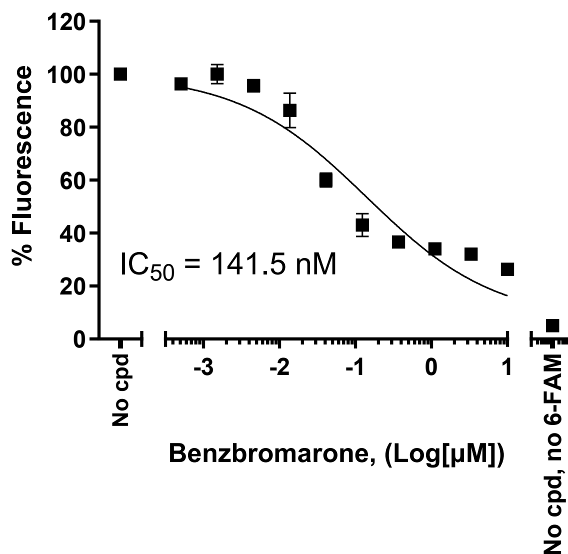

Figure 4. Inhibition of URAT1-mediated transport of 6-FAM by Benzbromarone in the URAT1 HEK293 Cell Line.

URAT1 HEK293 cells were treated with increasing concentrations of Benzbromarone along with 6-FAM for 1 hour. The uptake of 6-FAM was evaluated by measuring fluorescence intensity. Results are presented as percent of control, where the fluorescence intensity in 6-FAM treated cells in the absence of inhibitor compound was set at 100%.

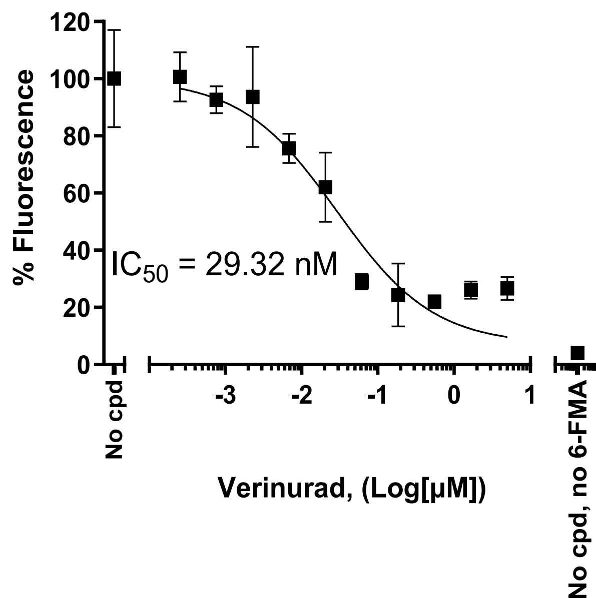

Figure 5. Inhibition of URAT1-mediated transport of 6-FAM by Verinurad in the URAT1 HEK293 Cell Line.

URAT1 HEK293 cells were treated with increasing concentrations of Verinurad along with 6-FAM for 1 hour. The uptake of 6-FAM was evaluated by measuring fluorescence intensity. Results are presented as percent of control, where the fluorescence intensity in 6-FAM treated cells in the absence of inhibitor compound was set at 100%.

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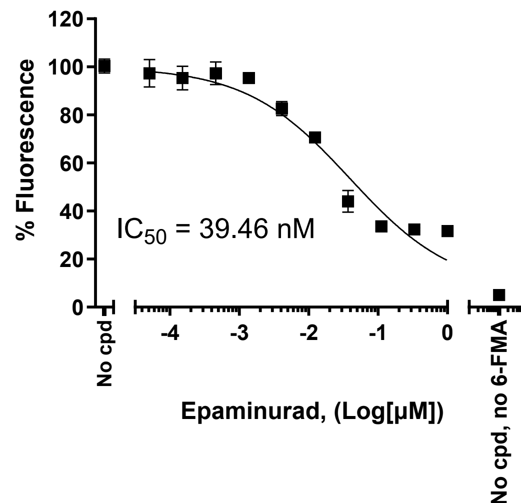

Figure 6. Inhibition of URAT1-mediated transport of 6-FAM by Epaminurad in the URAT1 HEK293 Cell Line.
URAT1 HEK293 cells were treated with increasing concentrations of Epaminurad along with 6-FAM for 1 hour. The uptake of 6-FAM was evaluated by measuring fluorescence intensity. Results are presented as percent of control, where the fluorescence intensity in 6-FAM treated cells in the absence of inhibitor compound was set at 100%.

Data shown is representative.

Sequence
Human URAT1 (SLC22A12) sequence (accession number NM_144585)

MAFSELLDLVGGLGRFQVLQTMALMVSIMWLCTQSMLENFSAAVPSHRCWAPLLDNSTAQASILGSLSPEALLAISIPPGPNQR
PHQCRRFRQPQWQLLDPNATATSWSEADTEPCVDGWVYDRSIFTSTIVAKWNLVCDSHALKPMAQSIYLAGILVGAAACGPAS
DRFGRRLVLTWSYLQMAVMGTAAAFAPAFPVYCLFRFLLAFAVAGVMMNTGTLLMEWTAARARPLVMTLNSLGFSFGHGLTA
AVAYGVRDWTLLQLVVSVPFFLCFLYSWWLAESARWLLTTGRLDWGLQELWRVAAINGKGAVQDTLTPEVLLSAMREELSMG
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ILANTLVPHEMGALRSALAVLGLGGVGAAFTCITIYSSELFPTVLRMTAVGLGQMAARGGAILGPLVRLLGVHGPWLPLLVYGTV
PVLSGLAALLLPETQSLPLPDTIQDVQNQAVKKATHGTLGNSVLKSTQF

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