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Data Sheet

DAT – CHOK1 Recombinant Cell Line
Cat #: 60558

Description
Recombinant CHOK1 cell line expressing Human sodium-dependent dopamine transporter (DAT), Genbank accession number NM_001044.

Background
The dopamine transporter (DAT, SLC6A3) is a membrane-spanning protein that pumps the neurotransmitter dopamine out of the synaptic cleft back into the cytosol, from which other transporters sequester DA and norepinephrine (NE) into vesicles for storage and later release. Dopamine reuptake via DAT provides the primary mechanism through which dopamine is cleared from synapses. DAT is implicated in a number of dopamine-related disorders, including attention deficit hyperactivity disorder, bipolar disorder, clinical depression, and alcoholism.

Application
- Monitor dopamine uptake activity
- Screen for activators or inhibitors of DAT activities in a cellular context

Host cell
CHO-K1 cells

Format
Each vial contains ~2 X 10^6 cells in 1 ml of 10% DMSO

Storage
Immediately upon receipt, store in liquid nitrogen.

Mycoplasma testing
The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

Thaw Medium 3 (BPS Bioscience #60186): F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

Growth Medium 3B (BPS Bioscience #79529): F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 500 µg/ml of Hygromycin B to ensure the recombinant expression is maintained.

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Cells should be grown at 37°C with 5% CO_2 using Growth Medium 3B.

DAT-CHO-K1 cells should exhibit a typical cell division time of 24 hours.

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 3 **(no Hygromycin)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 3 **(no Hygromycin)**. Transfer the resuspended cells to a T75 flask and incubate at 37°C in a 5% CO_2 incubator. After 24 hours of culture, add an additional 3 – 4 ml of Thaw Medium 3 **(no Hygromycin)**. At first passage, switch to Growth Medium 3B **(contains Hygromycin).**

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.25% Trypsin/EDTA, add Growth Medium 3B and transfer to a tube. Spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio: 1:5 to 1:10, twice a week.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.25% Trypsin/EDTA. Add Growth Medium 3B and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

Functional validation

Human DAT has been stably integrated into CHO-K1 cells and its constitutive expression was confirmed by Western blotting.

hDAT activity was confirmed by a fluorescence-based assay kit using a fluorescent substrate that mimics the biogenic amine neurotransmitters and is taken into the cell through the hDAT transporter. This results in increased intracellular fluorescence intensity that can be monitored in real time using a bottom-reading microplate reader. The hDAT enzymatic activity in hDAT-CHO-K1 cells can be blocked efficiently by a known hDAT specific inhibitor, Amfebutamone (Bupropion), as shown by the drop in the fluorescence increase rate.

Sample protocol to determine the effect of inhibitors on exogenously expressed hDAT in DAT-CHO-K1 cells:

Materials Required but Not Supplied

- Neurotransmitter Transporter Uptake Assay Kit (Molecular Devices #R6138)
- HBSS buffer (1×) (Hyclone #SH30588.01)
- Thaw Medium 3 (BPS Bioscience #60186)
- Growth Medium 3B (BPS Bioscience #79529)

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Note: We recommend each treatment be set up in at least triplicate.

1. On day 1, seed DAT-CHO-K1 cells at a density of 30,000-40,000 cells in 100 µl of Thaw Medium 3 into each well of a tissue culture-treated 96-well black with clear bottom plate. Incubate cells at 37˚C in a CO_2 incubator overnight. We recommend seeding a couple wells with CHO-K1 cells at the same density for use as a background control.

2. On day 2, removed plates from the incubator, aspirate the medium from the wells, and pipette 100 µL/well of testing compound diluted in 1 x HBSS Buffer to all wells. Incubated the plates at 37°C for 30 minutes to allow binding of the compound to the transporter.

3. Add 100 µL of Dye Solution per test well and transfer the assay plate directly to a bottom-read fluorescence microplate reader for 30 minutes on kinetic read-mode using excitation wavelength at 440 nm and emission wavelength at 520 nm.

Figure 1. Western Blot of hDAT expression in DAT-CHO-K1 cells. Western Blot of hDAT-CHO-K1 cells stained with rabbit anti-Dopamine transporter antibody (Millipore, #AB5802). The full length recombinant human DAT comprises 620 amino acids and has a calculated molecular mass of 68.5 kDa. It migrates as an approximately 85 kDa band in SDS-PAGE under reducing conditions.

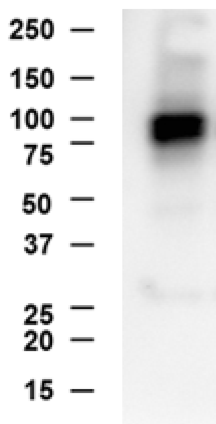

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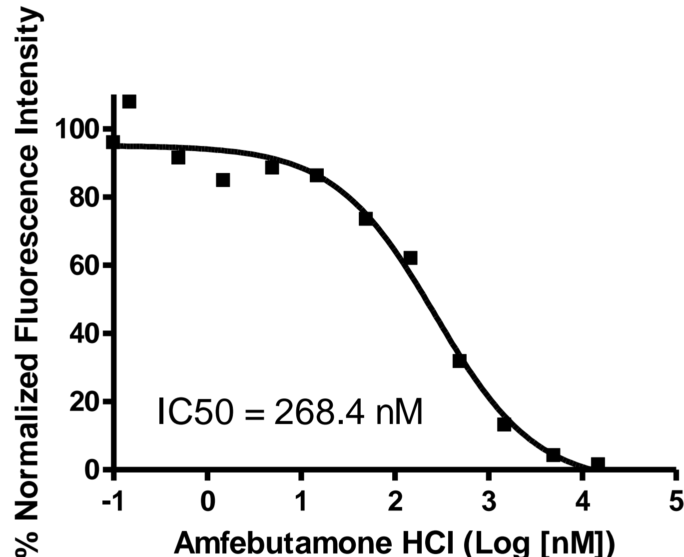
Figure 2. Dose response of hDAT activity in DAT-CHO-K1 cells to reference inhibitor Amfebutamone HCl. The IC50 of Amfebutamone HCl is ~ 268 nM.



Vector and sequence

Full length human DAT cDNA was cloned into a pIREShyg3 expression vector.

Polylinker: $P_{CMV\ IE}$-NheI-DAT-EcoRV-IVS-IRES-Hygromycin^R

hDAT sequence (accession number NM_001044)

MSKSKCSVGLMSSVVAPAKEPNAVGPKEVELILVKEQNGVQLTSSTLTNPRQSPVEAQ
DRETWGKKIDFLLSVIGFAVDLANVWRFPYLCYKNGGGAFLVPYLLFMVIAGMPLFYME
LALGQFNREGAAGVWKICPILKGVGFTVILISLYVGFFYNVIIAWALHYLFSSFTTELPWIH
CNNSWNSPNCSDAHPGDSSGDSSGLNDTFGTTPAAEYFERGVLHLHQSHGIDDLGPP
RWQLTACLVLVIVLLYFSLWKGVKTSGKVVWITATMPYVVLTALLLRGVTLPGAIDGIRA
YLSVDFYRLCEASVWIDAATQVCFSLGVGFGVLIAFSSYNKFTNNCYRDAIVTTSINSLT
SFSSGFVVFSFLGYMAQKHSVPIGDVAKDGPGLIFIIYPEAIATLPLSSAWAVVFFIMLLTL
GIDSAMGGMESVITGLIDEFQLLHRHRELFTLFIVLATFLLSLFCVTNGGIYVFTLLDHFAA
GTSILFGVLIEAIGVAWFYGVGQFSDDIQQMTGQRPSLYWRLCWKLVSPCFLLFVVVVS
IVTFRPPHYGAYIFPDWANALGWVIATSSMAMVPIYAAYKFCSLPGSFREKLAYAIAPEK
DRELVDRGEVRQFTLRHWLKV

References

1. Vaughan, RA., *et al., Trends in Pharmacological Sciences.* 2013; **34(9):** 489–496.
2. Jorgensen, S., *et al., Journal of Neuroscience methods.* 2008; **169:** 168-176.

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Related Products

Product	Cat. #	Size
Thaw Medium 3	60186	100 ml
SLC5A5 - HEK293 Recombinant Cell line	90333	2 vials
NET - CHOK1 Recombinant Cell Line	60557	2 vials
GRK6, GST-tag	40066	10 µg

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