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Data Sheet

NET – CHOK1 Recombinant Cell Line

Cat #: 60557

Description

Recombinant CHOK1 cell line expressing human norepinephrine transporter (NET), Genbank accession number NM_001043.

Background

NET is a monoamine transporter responsible for the sodium-chloride (Na^+/Cl^-)-dependent reuptake of extracellular norepinephrine (NE), also known as noradrenaline. NET can also reuptake extracellular dopamine (DA). The reuptake of these two neurotransmitters is essential in regulating concentrations in the synaptic cleft. NETs, along with the other monoamine transporters, play a key role in depression and neurodegenerative diseases (NDD) such as Alzheimer's and Parkinson's disease. NET is the target of many antidepressants and recreational drugs. In addition, an overabundance of NET is associated with ADHD.

Application

- Monitor norepinephrine uptake activity
- Screen for activators or inhibitors of NET 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.

Culture conditions

Thaw Medium 3 (BPS Cat. #60186): Ham's F-12 medium (Hyclone # SH30526.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

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Growth Medium 3B (BPS Cat. #79529): Thaw Medium 3 (BPS Cat. #60186), plus 500 µg/ml of Hygromycin B (Life Technologies # 10687-010) to ensure the recombinant expression is maintained.

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 3B (Thaw Medium 3 and Hygromycin B).

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 3 **(no Hygromycin B)**. Spin down cells, resuspend cells in pre-warmed Thaw Medium 3 **(no Hygromycin B)** and transfer the resuspended cells to a T25 flask and culture in 37°C in a CO_2 incubator overnight. The next day, replace the medium with fresh Thaw Medium 3 **(no Hygromycin B)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should reach ~80% confluence two days after being thawed. Cells should be split before they reach complete confluence. At first passage, switch to Growth Medium 3B **(Thaw Medium 3 plus Hygromycin B)**.

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: 1:10 twice a week .

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

hNET 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 hNET transporter. This results in increased intracellular fluorescence intensity that can be monitored in real time using a bottom-reading microplate reader. The hNET enzymatic activity in hNET-CHO-K1 cells can be blocked efficiently by a known hNET specific inhibitor, Duloxetine, as shown by the drop in the fluorescence increase rate.

Sample protocol to determine the effect of inhibitors on exogenously expressed hNET in NET-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 Cat. #60186)
- Growth Medium 3B (BPS Cat. #79529)

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

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1. On day 1, seed NET-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 hNET expression in NET-CHO-K1 cells. Western Blot of CHO-K1 (lane 1) and hNET-CHO-K1 cells (lane 2) stained with rabbit anti-Noradrenaline transporter antibody (Abcam, #AB84057). The full length recombinant human NET comprises 617 amino acids and has a calculated molecular mass of 65 kDa. It migrates as an approximately 60 kDa band in SDS-PAGE under reducing conditions.

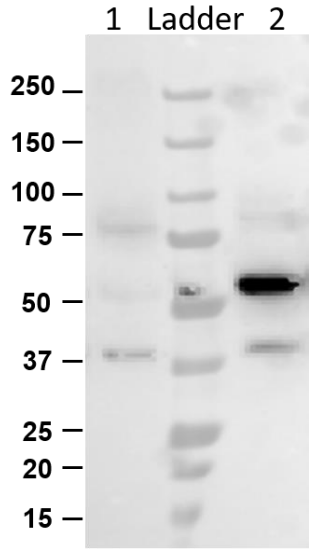

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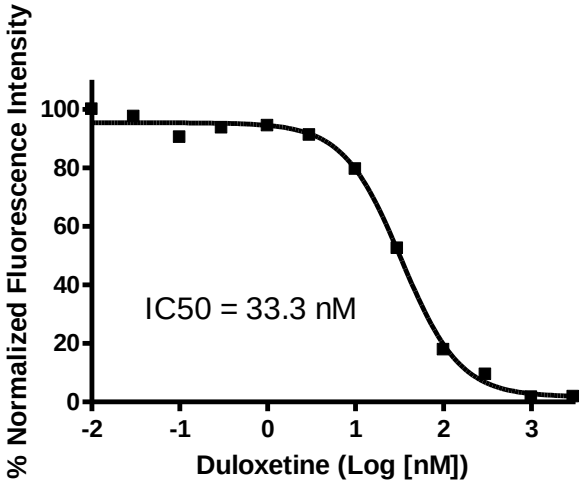
Figure 2. Dose response of hNET activity in NET-CHO-K1 cells to reference inhibitor Duloxetine HCl. The IC50 of Duloxetine is ~ 33.3 nM.



Vector and sequence
Full length human NET cDNA was cloned into a pIREShyg3 expression vector.

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

hNET sequence (accession number NM_001043)

MLLARMNPQVQPENNGADTGPEQPLRARKTAELLVVKERNGVQCLLAPRDGDAQPRE
TWGKKIDFLLSVVGFAVDLANVWRFPYLCYKNGGGAFLIPYTLFLIIAGMPLFYMELALG
QYNREGAATVWKICPFFKGVGYAVILIALYVGFYYNVIIAWSLYYLFSSFTLNLPWTDCG
HTWNSPNCTDPKLLNGSVLGNHTKYSKYKFTPAAEFYERGVLHLHESSGIHDIGLPQW
QLLLCLMVVVIVLYFSLWKGVKTSGKVVWITATLPYFVLFVLLVHGVTLPGASNGINAYL
HIDFYRLKEATVWIDAATQIFFSLGAGFGVLIAFASYNKFDNNCYRDALLTSSINCITSFV
SGFAIFSILGYMAHEHKVNIEDVATEGAGLVFILYPEAISTLSGSTFWAVVFFVMLLALGL
DSSMGGMEAVITGLADDFQVLKRHRKLFTFGVTFSTFLLALFCITKGGIYVLTLLDTFAA
GTSILFAVLMEAIGVSWFYGVDRFSNDIQQMMGFRPGLYWRLCWKFVSPAFLLFVVVV
SIINFKPLTYDDYIFPPWANWVGWGIALSSMVLVPIYVIYKFLSTQGSLWERLAYGITPEN
EHHLVAQRDIRQFQLQHWLAI

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1. Zhou, J., *Drugs Future.* 2004; **29(12):** 1235–1244.
2. Jorgensen, S., *et al., Journal of Neuroscience methods.* 2008; **169:** 168-176.

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