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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Description

DLL4 CHO Cell Line is a CHO-K1 cell line expressing human DLL4 (delta-like 4) (accession number NM_019074.2) under the control of the cytomegalovirus (CMV) promoter. This cell line was generated by lipid-mediated transfection followed by hygromycin selection and limiting dilution. Individual clones were screened for DLL4 expression by flow cytometry in order to obtain this DLL4 expressing cell line.

Background

Notch signaling is an evolutionarily conserved pathway that plays a critical role in cell fate decisions and differentiation. A member of the Delta family of Notch ligands, DLL4 (delta-like 4) is a vascular transmembrane protein which signals through its corresponding receptors Notch1 and Notch4 and plays a critical role in embryonic development, where it is essential for both vascular development and T-cell specification. DLL4 expression is induced by both VEGF (vascular endothelial growth factor) and hypoxia. It plays an important role in blood vessel growth by regulating the differentiation of tip and stalk cells directing vascular sprouting. Early studies of the role of DLL4-Notch signaling in tumor angiogenesis demonstrated that inhibition of DLL4 decreased tumor growth. DLL4 is an attractive therapeutic target with several clinical trials of DLL4 inhibitors ongoing, including trials of bispecific antibodies targeting both DLL4 and VEGF.

Application

Screen antibodies against DLL4.

Materials Provided

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

Parental Cell Line

CHO-K1 cells, Chinese Hamster Ovary, epithelial-like cells, adherent

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 3	BPS Bioscience #60186
Growth Medium 3B	BPS Bioscience #79529

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% CO₂. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

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.

Cell Culture Protocol

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 3.

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 3.
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO₂ incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 and continue growing in a 5% CO₂ 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 3B.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca²⁺/Mg²⁺, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3B 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 3B.

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

Cell Freezing

- Aspirate the medium, wash the cells with PBS without $\text{Ca}^{2+}/\text{Mg}^{2+}$, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
- Once the cells have detached, add Growth Medium 3B and count the cells.
- Spin down the cells at $300 \times g$ for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at $\sim 2 \times 10^6$ cells/ml.
- 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.
- 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.

A. Validation Data

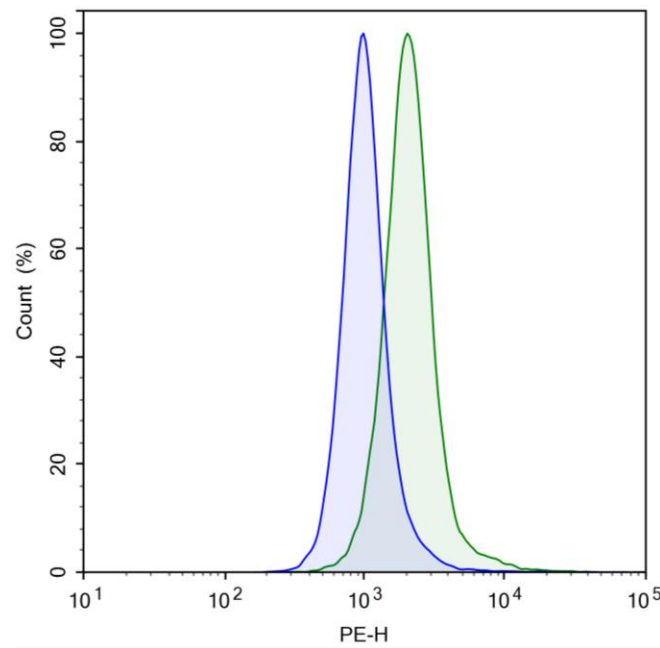


Figure 1: Cell surface expression of DLL4 in DLL4 CHO Cell Line by flow cytometry.

DLL4 CHO cell line (green) and control parental CHO-K1 cells (blue) were stained with PE anti-human Delta-like protein 4 (DLL4) Antibody (Biolegend #346506) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates PE intensity.

Sequence

Human DLL4 sequence (accession number NM_019074.2)

MAAASRSASGWALLLLVALWQQRAAGSGVFQLQLQEFINERGVLASGRPCEPGCRTFFRVCLKHFQAVVSPGPCTFGTVSTPVL
GTNSFAVRDDSSGGGRNPLQLPFNFTWPGTFSLIIEAWHAPGDDLRLPEALPPDALISKIAIQGSLAVGQNWLLDEQTSTLTRLRYS
YRVICSDNYYGDNCSRLCKKRNDHFGHYVCQPDGNLSCLPGWTGEYCQQPICLSGCHEQNGYCSKPAECLCRPGWQGRLCNEC
IPHNGCRHGTCSTPWQCTCDEGWGGLFCDQDLNYCTHHSPCKNGATCSNSGQRSYTCTCRPGYTGVDCELESECDNSNPCRN
GGCKDQEDGYHCLCPPGYGLHCEHSTLSCADSPCFNGGSCRERNQGANYACECPPNFTGSNCEKKVDRCTS NPCANGGQCL
NRGPSRMCRCPGFTGT YCELHVSDCARNPCAHHGGTCHDLENGLMCTCPAGFSGRRCEVRTSIDACASSPCFN RATCYTDLSTD
TFVCNCPYGFVGSRCFVPVGLPPSFPWVAVSLGVGLAVLLVLLGMVAVAVRQLRLRRPDDGSREAMNNLSDFQKDNLIPAAQLK
NTNQKKELEVDCGLDKSNCGKQQNHTLDYNLAPGPLGRGTM PGKFPHSDKSLGEKAPLRLHSEKPECRISAICSPRDSMYQSVCL
ISEERNECVIATEV

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Notch1 Pathway Reporter Kit (Human)	79503	500 reactions
Notch1dE Lentivirus	78747	500 µl x 2
Notch1/CSL Luciferase Reporter HEK293 Cell Line	60652	2 Vials
DLL3 CHO Cell Line	78882	2 Vials

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