Description

DLL3 CHO Cell Line is a CHO-K1 cell line expressing human DLL3 (delta like canonical Notch ligand 3) (accession number NM_016941.3) under the control of the cytomegalovirus (CMV) promoter. This cell line was generated by lentiviral transduction followed by puromycin selection and limited dilution. Individual clones were screened based on DLL3 expression to obtain this high-expressing cell line.

This cell line has been validated by flow cytometry and in a co-culture assay with a bispecific molecule.

Background

DLL3, also known as delta like ligand three, is a Notch ligand characterized by a DSL domain, transmembrane region, and a series of EGF repeats. Notch ligands can participate in trans-interactions (interaction with Notch receptor on a different cell) and cis interactions (interaction with Notch receptor within the same cell) to activate or inhibit Notch signaling, respectively. DLL3 exclusively functions to inhibit Notch signaling through cis inhibition. While DLL3 expression is limited in healthy tissue, high expression levels of DLL3 are found in various cancers including small cell lung cancer (SCLC), where it plays an oncogenic role. Relieving DLL3-mediated inhibition of Notch signaling may serve as a therapeutic avenue, with drugs being developed to target DLL3 as a possible lung cancer therapy (example: rovalpituzumab tesirine).

Application

- Use as target cells to validate and characterize small molecule drugs directed at DLL3.
- Perform CAR-T studies.

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

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 3L	BPS Bioscience #78104

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Anti-DLL3 Antibody, PE-Labeled	BPS Bioscience #102503
NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #60621
CHO-K1 Cell Line	ATCC #CCL-61
Anti-DLL3-Anti-CD3 Bispecific Molecule	BPS Bioscience #102209
96-well tissue culture-treated white clear-bottom assay plate	
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

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.

Media Required for Cell Culture

Thaw Medium 3 (BPS Bioscience #60186):

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3L (BPS Bioscience #78104):

F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 6 µg/ml of Puromycin.

Media Required for Cellular Assay:

Thaw Medium 2 (BPS Bioscience #60184):

RPMI 1640 (ATCC modification) medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

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% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 and continue cell 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 3L**.**

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 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 3L 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 3L.

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

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 3L 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.

A. Validation Data

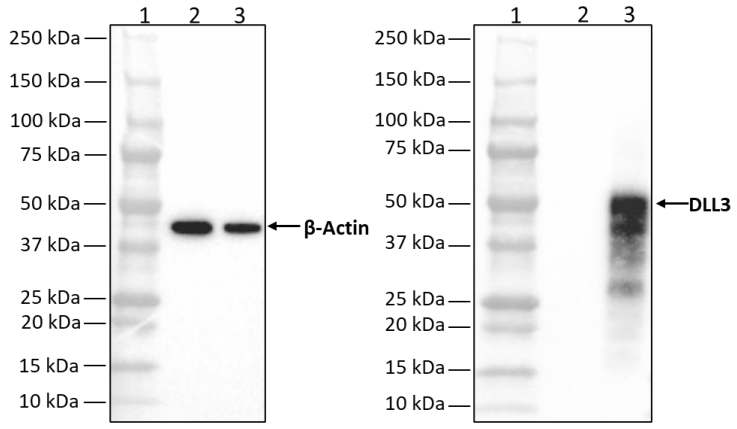

Figure 1. Western Blot analysis of DLL3 protein expression in DLL3 CHO cells.
DLL3 CHO cell lysate was analyzed by Western Blot using either Rabbit Anti-DLL3 (Abcam #ab229902) or Rabbit Anti-Actin (Cell Signaling Technology #4970) primary antibodies, and Anti-Rabbit (SBCT #2357) HRP-conjugated antibody as secondary antibody.

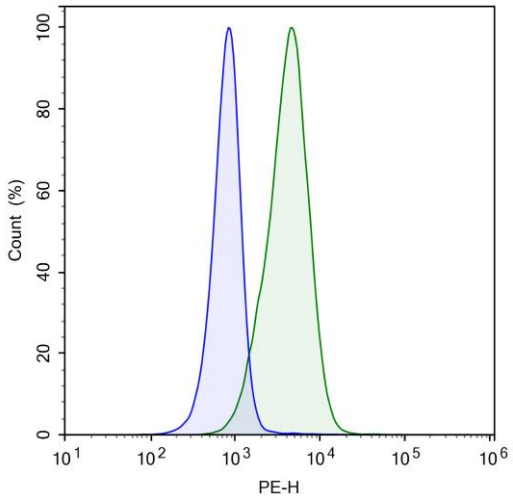

Figure 2: Flow cytometry analysis of DLL3 cell surface expression in DLL3 CHO Cell Line.
DLL3 CHO cell line (green) and control parental CHO-K1 cells (blue) were stained with Anti-DLL3 Antibody, PE-Labeled (#102503) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates PE intensity.

B. Functional characterization of DLL3 CHO Cell Line in a co-culture assay with NFAT Luciferase Reporter Jurkat Cell Line in the presence of Anti-DLL3-Anti-CD3 Bispecific Molecule

Assay Medium: Thaw Medium 2 (#60184)

- All conditions should be performed in triplicate.
- The assay should include "Luminescence Background Control" and "Test Condition".
- CHO-K1 Cell Line can be used a control.

1. Seed CHO and DLL3 CHO cells at 30,000 cells/well in volume of Assay Medium and allow a few hours for the cells to attach in a 96-well clear bottom white plate. Leave a few empty wells as Luminescence Background Control.

2. Prepare a cell suspension of NFAT Luciferase Reporter Jurkat cells (30,000 cells/ well).

3. Add 30,000 cells/well of NFAT Luciferase Reporter Jurkat cells.

4. Prepare a 1:4 serial dilution of Anti-DLL3-Anti-CD3 Bispecific Molecule in Assay Medium, starting from 100 nM (50 µl volume/ well). The bispecific antibody simultaneously binds to TCR/CD3 on the NFAT Luciferase Reporter Jurkat cells and the tumor antigen DLL3 on DLL3 CHO cells.

5. Add 50 µl of serial diluted bispecific molecule to the "Test Condition" wells.

6. Add 50 µl of Assay Medium to the "Luminescence Background Control" wells.

7. After 16 hours the luciferase activity is measured using ONE-Step™ Luciferase Assay System (#60690) per recommended protocol.

8. Measure luminescence using a luminometer.

9. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells.

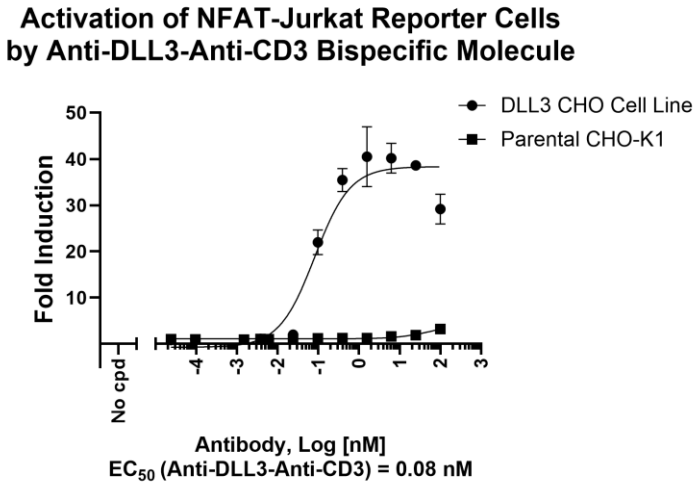

Figure 3: Activation of NFAT Reporter Jurkat Cell Line by Anti-DLL3-Anti-CD3 Bispecific Molecule when co-cultured with DLL3 CHO Cell Line.
DLL3 CHO cells were co-cultured with NFAT Luciferase Reporter Jurkat cells in the presence of Anti-DLL3-Anti-CD3 Bispecific Molecule. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. The results are shown as fold induction.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Sequence
Human DLL3 sequence (accession number NM_016941.3)

MVSPRMSGLLSQTVILALIFLPQTRPAGVFELQIHSFGPGPGPGAPRSPCSARLPCRLFFRVCLKPGLSEEAAESPCALGAA
LSARGPVYTEQPGAPAPDLPLPDGLLQVPFRDAWPGTFSFIIETWREELGDQIGGPAWSLLARVAGRRRLAAGGPWAR
DIQRAGAWELRFSYRARCEPPAVGTACTRLCRPRSAPSRCGPGLRPCAPLEDECEAPLVCRAGCSPEHGFCEQPGECRCL
EGWTGPLCTVPVSTSSCLSPRGPSSATTGCLVPGPGPCDGNPCANGGSCSETPRSFECTCPRGFYGLRCEVSGVTCADGP
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ACANGGTCVEGGGAHRCSCALGFGGRDCRERADPCAARPCAHGGRCYAHFSGLVCACAPGYMGARCEFPVHPDGAS
ALPAAPPGLRPGDPQRYLLPPALGLLVAAGVAGAALLLVHVRRRGHSQDAGSRLLAGTPEPSVHALPDALNNLRTQEGS
GDGPSSSVDWNRPEDVDPQGIYVISAPSIYAREVATPLFPPLHTGRAGQRQHLLFPYPSSILSVK

References

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Ladi E., *et al*., 2005 *J Cell Biol*. 170 (6): 983–992.
Kunnimalaiyaan M., *et al.,* 2007 *The oncologist*. 12(5):535-42.
Owen D., *et al*., 2019 *J Hematol Oncol. 12*(1): 61.

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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

Products	*Catalog #*	*Size*
Human Notch1 Pathway Reporter Kit	79503	500 reactions
Mouse Notch1 Pathway Reporter Kit	60509	500 reactions
Mouse Notch1 NICD Pathway Reporter Kit	79504	500 reactions
CSL Reporter-HEK293 Cell Line	79754	2 vials

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