6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Data Sheet

CD137L (4-1BBL) CHO-K1 Recombinant Cell Line
Catalog # 60523

Background
CD137L (4-1BBL; TNLG5A), a part of the tumor necrosis factor (ligand) superfamily (TNFSF9), is a type II glycoprotein that expresses on various antigen-presenting cells. It interacts with CD137 (4-1BB) on T cells following TCR stimulation to promote T cell expansion and cytokine production.

Description
Recombinant CHO-K1 cell constitutively expressing full-length human CD137L (NP_003802.1). Surface expression is confirmed by flow cytometry.

Application
This cell line is useful to stimulate human CD137 (4-1BB) on immune cells, and for use in binding assays for CD137.

Host Cell
Chinese Hamster Ovary Cells. Adherent epithelial cells.

Format
Each vial contains ~ 2 x 10^6 cells in 1mL of 10% DMSO in FBS.

Storage
Store in liquid nitrogen immediately upon receipt.

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

Growth Medium 3D (BPS Bioscience, #79539): F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml G418

Recommended Culture Condition
Prepare a 50 ml conical tube and a T-25 culture flask with 5 ml of pre-warmed Thaw Medium 3. Quickly thaw cells in a 37°C water bath with constant and slow agitation. Clean the outside of the vial with 70% ethanol and immediately transfer the entire contents to the conical tube with Thaw Medium 3 **without G418** and rock the tube the tube gently. Centrifuge the cells at 200 x g for 3 minutes. Re-suspend the cells in 5 ml of pre-warmed Thaw Medium 3 and transfer the entire contents to the T25 culture flask containing Thaw Medium 3 **without G418.** Avoid pipetting up and down, and gently rock the flask to distribute the cells. Incubate the cells in a

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humidified 37°C incubator with 5% CO_2. After 24-48 hours incubation, change to fresh medium without disturbing the attached cells. Continue to change the Thaw Medium 3 **without G418** every 2-3 days until reached desired confluency. If slow cell growth occurs during resuscitation, increase the FBS to 15% during the first week of culture.

Subculture: When cells reached 90% confluency, remove the media and wash twice with PBS (without Magnesium or Calcium). Treat cells with 2-3 ml of 0.25% trypsin/EDTA and incubate for 2-3 minutes at 37°C. After confirming cell detachment by a light microscope, add 10mL of pre-warmed Growth Medium 3D **(with G418)**, and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 ml conical tube and centrifuge at 200xg for 5 minutes. Remove the media and resuspend cells in 10 ml of pre-warmed Growth Medium 3D **(with G418)**. Dispense 2 mL of the cell suspension into a new T75 flask containing pre-warmed 18 ml Growth Medium 3D **(with G418)**. Incubate cells in a humidified incubator as described above.

Freeze cells in freezing media (10% DMSO in FBS) when they reach 90% confluency. It is not recommended to use the cells after passage 20.

Mycoplasma Testing
This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (catalogue #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (catalogue #LT07-518) was used as a positive control.

Application References
1. Yoshimori, M., *et al.* (2014) CD137 Expression Is Induced by Epstein-Barr Virus Infection Through LMP1 in T or NK Cells and Mediates Survival Promoting Signals. *PLoS One* **9:** e112564.
2. Kim, H.J., *et al.* (2012) Reverse Signaling Through the Costimulatory Ligand CD137L in Epithelial Cells is Essential for Natural Killer Cell-Mediated Acute Tissue Inflammation. *Proc. Natl. Acad. Sci.* **109:** E13-E22
3. Salih, H.R., *et al.* (2000) Constitutive Expression of Functional 4-1bb (CD137) Ligand on Carcinoma Cells. *J Immunology* **165:** 2903-2910.

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Quality Assurance

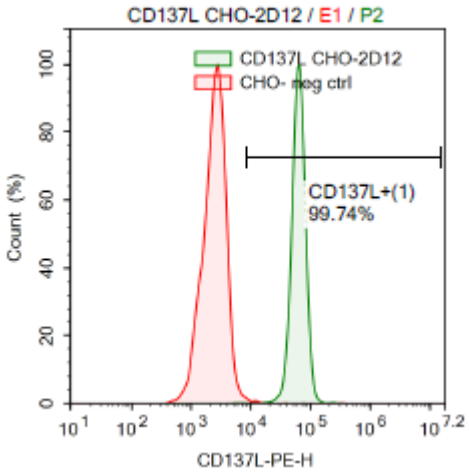

Figure 1. Human CD137L expression in CHO-K1 cells
Flow cytometry demonstrated PE-conjugated anti-human CD137L antibody (Clone 5F4; Biolegend, Cat. # 311503) detects CD137L-positive cells (green), but not CHO-K1 wild-type cells used as a negative control (red).

Vector and Sequence
Human CD137L (NM_003811.3) was cloned into the MCS of pIRESneo3 vector (Clontech, Cat. #631621).

AA Sequence
MEYASDASLDPEAPWPPAPRARACRVLPWALVAGLLLLLLLAAACAVFLACPWAVSGARASP
GSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLS
YKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPAS
SEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSPR
SE

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Related Products	**Cat. #**	**Size**
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
ONE-Step™ Luciferase Assay System	60690-3	1 L
Thaw Media 3	60186	100 ml
Human CD137, Fc fusion (hIgG1)	71170	100 µg
Human CD137, Fc fusion (hIgG1), biotinylated	71171	50 µg
Mouse CD137, Fc fusion (hIgG1)	71250	100 µg
Mouse CD137, Fc fusion (mIgG2a)	71254	100 µg
Mouse CD137, Fc fusion (mIgG2a) , biotinylated	71255	50 µg

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