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Data Sheet

CD137 (4-1BB) HEK293 Recombinant Cell Line
Catalog # 60691

Background
Human CD137 (4-1BB; TNFRS9) is an inducible costimulatory molecule that activates T cells. CD137-CD137L mediated signaling has been shown to be important for proliferation, effector functions and survival of T cells. CD137 is also expressed in NK and NKT cells. Antibodies targeting CD137 activation in immune cells have demonstrated potent anti-tumor effects in cancer patients.

Description
Recombinant HEK293 cell constitutively expressing full length human CD137 (Genbank #NM_001561). Surface expression is confirmed by flow cytometry.

Host Cell
Human Embryonic Kidney cell line (HEK293). Adherent epithelial cells.

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

Storage
Store in liquid nitrogen immediately upon receipt.

Culture Medium

Thaw Medium 1 (BPS, #60187): MEM medium (Hyclone, #SH30024.01) supplemented with 10% FBS (Invitrogen, #26140-079), 1% non-essential amino acids (Hyclone, #SH30238.01), 1 mM Na pyruvate (Hyclone, #SH30239.01), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01)

Growth Medium 1F (BPS, #79540)**:** Thaw Medium 1 (BPS, #60187) plus 100 µg/ml Hygromycin B (Thermo Fisher, #10687010).

Recommended Culture Condition
Frozen Cells: Prepare a 50 ml conical tube with 10 ml of pre-warmed Thaw Medium 1 (**no hygromycin**). 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 content to Thaw Medium 1 (**no hygromycin**). Avoid pipetting up and down, and gently rock the conical tube.

Spin the cells down at 150 x g for 5 minutes. Discard the medium and re-suspend the cell pellet in fresh Thaw Medium 1 (**no hygromycin**). Transfer the entire content to a T25 flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO_2. After 48-72 hours of incubation, change to fresh Thaw Medium 1 (**no hygromycin**), without disturbing the

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attached cells. Continue to change the medium every 2-3 days until the cells reach desired confluency. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture. Begin adding Hygromycin B to Thaw Medium 1 (Growth Medium 1F) after the first passage.

Subculture: When cells reach 90% confluency, remove the medium and GENTLY wash once with PBS (without Magnesium or Calcium). These cells are loosely adherent and detach easily so do not re-suspend the PBS directly onto the cell surface. Treat cells with 2 ml of 0.25% trypsin/EDTA and incubate for 2-3 minutes at 37°C. After confirming cell detachment by light microscopy, add 10 ml pre-warmed medium and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes. Remove the medium and re-suspend cells in 10 ml of pre-warmed Growth Medium 1F. Dispense 5 ml of the cell suspension into a new T75 flask containing 20 ml pre-warmed media. Incubate cells in a humidified 37°C incubator with 5% CO_2. Freeze cells in freezing medium (10% DMSO in FBS) when cells reach 90% confluency. Cells have been demonstrated to be stable for at least 15 passages; BPS recommends preparing frozen stocks so cells are not used beyond passage 20.

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

Application
This cell line is useful for CD137 binding assays or downstream CD137-dependent signaling events.

Application References
1. McNamara II, J.O., *et.al.* (2008) Multivalent 4-1BB binding aptamers co-stimulate CD8+ T cells and inhibit tumor growth in mice. *J. Clin. Invest*. **118:** 376-386.
2. Ma, B.Y., *et.al.* (2005) The expression and regulatory role of OX40 and 4-1BB heterodimers in activated human T cells. *Blood*. **106:** 2002-2010.

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Quality Assurance

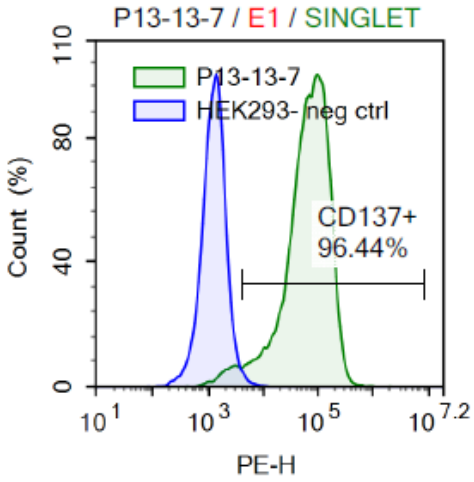

Figure 1. Expression of CD137 (4-1BB) protein validated by flow cytometry. Flow cytometry showed PE-conjugated anti-human CD137 antibody (Clone 4B4-1, Biolegend, #309803) detects CD137 (4-1BB)-positive population (green), using wild-type HEK293 cells as a negative control (blue).

Vector and Sequence
Human CD137 (NM_001561) was cloned into pIREShyg.

MGNSCYNIVATLLLVLNFERTRSLQDPCSNCPAGTFCDNNRNQICSPCPPNSFSSAGGQRTCD
ICRQCKGVFRTRKECSSTSNAECDCTPGFHCLGAGCSMCEQDCKQGQELTKKGCKDCCFGT
FNDQKRGICRPWTNCSLDGKSVLVNGTKERDVVCGPSPADLSPGASSVTPPAPAREPGHSPQ
IISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGG
CEL

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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
CD137L (4-1BBL) CHO-K1 Cell Line	60523	2 vials
CD137L (4-1BBL) / TCR Activator- CHO-K1 Cell Line	60527	2 vials
CD137[Biotinylated]:CD137L Inhibitor Screening Assay Kit	72025	96 rxns.
CD137, Fc fusion (mIgG2a), Biotin-labeled (Mouse) HiP™	71255	50 µg
CD137, Fc fusion (mIgG2a), Avi-tag (Mouse) HiP™	71254	100 µg
CD137, Fc fusion (hIgG1) (Mouse)	71250	100 µg
CD137, Fc Fusion (Human) HiP™	71170	100 µg
CD137, Fc Fusion, Biotin-labeled (Human) HiP™	71171	50 µg
CD137L (4-1BB ligand), His-tag	71189	100 µg
Thaw Medium 1	60187	100 ml