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Data Sheet

CD137/NF-κB Reporter - HEK293 Recombinant Cell Line
Catalog # 79289

Background
Human CD137 (4-1BB; TNFRS9) is an inducible co-stimulatory molecule that activates T cells. CD137:CD137L-mediated signaling has been shown to be important for proliferation, effector functions and survivals of T cells. CD137 is also expressed in NK and NKT cells. Accumulating evidence shows a role for CD137:CD137L signaling in inflammation, suggesting that inhibition of this pathway may provide a therapeutic avenue to treat autoimmune and inflammatory diseases. Similarly, antibodies targeting CD137 activation in immune cells have demonstrated potent anti-tumor properties in cancer patients.

Description
Recombinant HEK293 cell line expressing a full length human CD137 (NM_001561). The NF-κB luciferase reporter construct is stably integrated into the genome. The firefly luciferase gene is controlled by 4 copies of NF-κB response element located upstream of the TATA promoter. Following activation by human CD137 ligand, NF-κB transcription factors bind to the DNA response elements to induce transcription of the luciferase gene.

Applications
- Screen for activators or inhibitors of CD137 signaling in a cellular context
- Characterize the biological activity of CD137 and its interactions with ligands and antibodies

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

Mycoplasma Testing
This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination.

Storage
Store in liquid nitrogen immediately upon receipt.

Culture Medium:

Thaw Medium 1 (BPS Bioscience, #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Thermo Fisher, Cat. #26140079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01).
Growth Medium 1G (BPS Bioscience, #79544): Thaw Medium 1, 400 µg/ml of Geneticin (Thermo Fisher, Cat. #11811031) and 50 µg/ml of Hygromycin B (Thermo Fisher, Cat. #10687010).

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Recommended Culture Condition

Frozen Cells: Prepare a 50 ml conical tube with 10 ml of pre-warmed Thaw Medium 1 (no G418 or 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 G418 or 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 G418 or 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 24 hours of incubation, change to fresh Thaw Medium 1 (no G418 or hygromycin), being careful to not disturb the attached cells. Begin adding G418 and Hygromycin B to Thaw Medium 1 (Growth Medium 1G) after the first passage.

Subculture: When cells reach 90% confluency, remove the medium and gently wash once with PBS (without Magnesium or Calcium). Treat cells with 0.5 ml of 0.05% trypsin/EDTA and incubate for 2-3 minutes at 37°C. Add 10 ml pre-warmed Growth Medium 1G 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 1G. Dispense 5 ml of the cell suspension into a new T75 flask containing 10 ml pre-warmed Growth Medium 1G. 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.

Additional reagents required for this assay:

CD137L protein (BPS Bioscience, #71189)
Anti-CD137 Agonist Antibody (BPS Bioscience, #79097-2)
ONE-Step Luciferase Detection Reagent (BPS Bioscience, #60690)
Thaw Medium 1 (BPS Bioscience, #60187)
Growth Medium 1G (BPS Bioscience, #79544)

Assay Protocol

1. Harvest CD137/NF-κB reporter-HEK293 cells from culture in Growth Medium 1G and seed cells at a density of ~35,000 cells per well into a white clear-bottom 96-well microplate in 90 µl of Thaw medium 1. Incubate the plate at 37°C in a CO_2 incubator.

2. 24 hours after seeding, serially dilute the CD137L protein and anti-CD137 agonist antibody in Thaw Medium 1. For an EC50 curve, we recommend a range of approximately 1 ng/ml to 10 µg/ml, final concentration.

   Set up each treatment in at least triplicate
   Add 10 µl diluted CD137L to the treated wells.
   Add 10 µl Thaw Medium 1 to control wells.
   Add 100 µl Thaw Medium 1 to cell-free control wells (for determining background luminescence)

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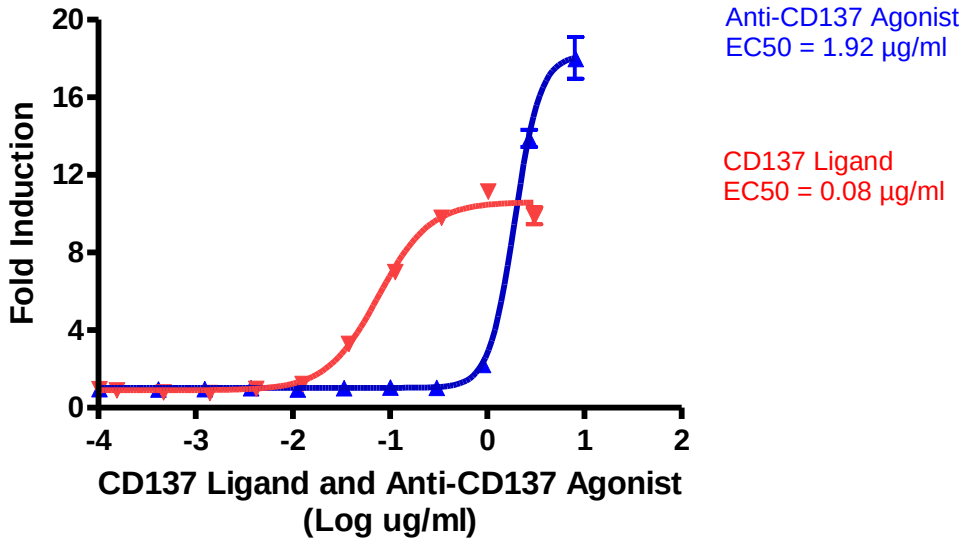
3. Incubate the plate at 37°C in a CO_2 incubator for ~ 6 hours for CD137L and 24 hrs for anti-CD137 agonist antibody.

4. Perform luciferase assay by using the ONE-Step luciferase assay system: Briefly, add 100 µl of One-Step Luciferase reagent per well and rock at room temperature for ~30 minutes. Measure luminescence using a luminometer.

5. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NF-κB luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

Figure 1. Dose response of CD137/ NF-κB-reporter HEK293 cells

CD137 ligand (BPS Bioscience, #71189) and anti-CD137 agonist (BPS Bioscience, #79097) were diluted and added to the cells (BPS Bioscience, #79289), then incubated at 37°C cell culture incubator for 6 and 24 hrs. respectively. After the treatment, perform Luciferase assay using One-Step Luciferase assay system (BPS Bioscience, #60690)



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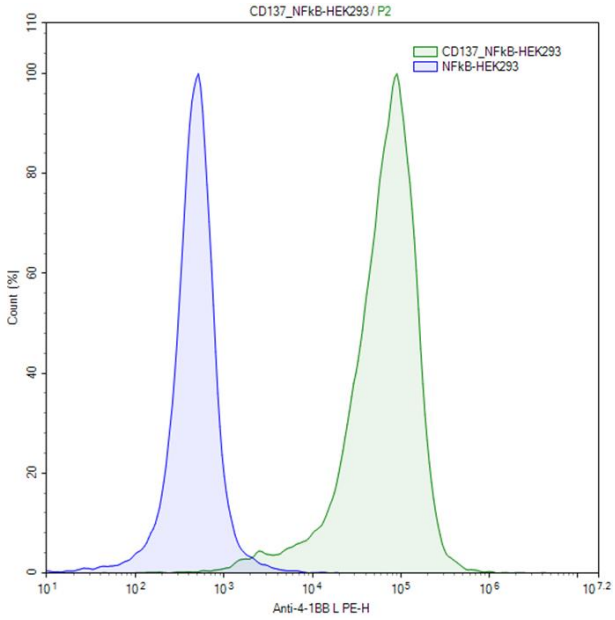
Quality Assurance

Figure 2. Expression of CD137 (4-1BB) protein validated by flow cytometry. Flow cytometry showed PE-conjugated anti-human 4-1BB antibody (Clone 4B4-1, Biolegend, #309803) detects 4-1BB expression cells (green), using wild-type HEK293 cells as a negative control (blue).



Application References

1. McNamara II JO *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 BY *et.al.* (2005) The expression and regulatory role of OX40 and 4-1BB heterodimers in activated human T cells. *Blood*. **106:** 2002-2010.

Vector and Sequence

Human CD137 (4-1BB), GenBank Accession #NM_001561, was cloned into pIREShyg3.

MGNSCYNIVATLLLVLNFERTRSLQDPCSNCPAGTFCDNNRNQICSPCPPNSFSSAGGQRTCD
ICRQCKGVFRTRKECSSTSNAECDCTPGFHCLGAGCSMCEQDCKQGQELTKKGCKDCCFGT
FNDQKRGICRPWTNCSLDGKSVLVNGTKERDVVCGPSPADLSPGASSVTPPAPAREPGHSPQ
IISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGG
CEL

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Related Products	Cat. #	Size
Anti-CD137 Agonist Antibody	79097-2	100 µg
CD137L (4-1BBL) CHO-K1 Recombinant Cell Line	60523	2 vials
CD137 (4-1BB) HEK293 Recombinant Cell Line	60691	2 vials
NF-κB Reporter (Luc)-HEK293 cell line	60650	2 vials
CD137L, His-tag	71189	100 µg
CD137, Fc fusion (mIgG2a), Avi-tag (Mouse) HiP™	71254	100 µg
CD137, Fc fusion (mIgG2a), Biotin-labeled (Mouse) HiP™	71255	50 µg
CD137, Fc fusion (hIgG1) (Mouse)	71250	100 µg
CD137 (4-1BB), Fc fusion (Human) HiP™	71170	100 µg
CD137, Fc fusion (mIgG2a), Biotin-labeled (Mouse) HiP™	71171	50 µg
CD137L, His-Tag (Mouse)	72517	100 µg
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
CD137[Biotinylated]:CD137L Inhibitor Screening Kit	72025	96 rxns
Thaw Medium 1	60187	100 ml