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Data Sheet

HVEM - HEK293 Recombinant Cell Line

Cat #: 79313

Product Description
Recombinant HEK293 stably expressing human HVEM (Herpesvirus entry mediator; CD270; TNFRSF14; ATAR) GenBank Accession # NM_003820).

Background
HVEM (CD270, TNFRSF14) is a human cell surface receptor in the TNF-receptor superfamily that can act as both a co-stimulatory receptor and a co-inhibitory receptor of T cells. Binding of HVEM to one of its ligands, LIGHT (CD258, TNFSF14) or LTα (lymphotoxin-α), causes a co-stimulatory signal which can activate lymphoid cells. Alternately, interaction with BTLA (CD272) or CD160 causes a co-inhibitory signal which negatively regulates T-cell immune responses. HVEM has also been shown to interact with adaptor proteins TRAF2 and TRAF5, and is critical to herpes simplex virus (HSV) cellular entry.

Application
- HVEM binding molecule (such as anti-HVEM antibody) screening and profiling in a cellular context
- Binding studies using HVEM ligands

Format
Each vial contains ~3 X 10^6 cells in 1 ml of 10% DMSO

Storage
Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing
The cell line has been screened using the metabolite-based Mycoplasma Detection Kit (Biotool #B3903) to confirm the absence of Mycoplasma species.

General Culture Conditions

Thaw Medium 1 (BPS Bioscience, #60187): MEM medium (Hyclone, #SH30024.01) + 10% FBS (Life Technologies, #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 Bioscience, #79540): Thaw Medium 1 (BPS Bioscience, #60187) plus 100 μg/ml of Hygromycin B (Life Technologies, #10687-010).

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Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1F to ensure recombinant expression. HVEM-HEK293 cells should display a typical cell division time of about 24 hours.

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 **(no Hygromycin B)**, spin down cells at 1000 rpm and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 **(no Hygromycin)**. Transfer resuspended cells to a T25 flask and culture at 37°C in a 5% CO_2 incubator overnight. The next day, replace the medium with fresh warm Thaw Medium 1 **(no Hygromycin B)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At first passage switch to Growth Medium 1F **(contains Hygromycin B)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS) and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Growth Medium 1F (**contains Hygromycin B)** and transfer to a tube, spin down cells, resuspend cells in Growth Medium 1F (**contains Hygromycin B)** and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ratio: 1:5 to 1:10 weekly or twice a week.

Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~ 1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split with a higher ratio.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 1 (**no Hygromycin B)** and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage.

It is recommended to expand the cells and freeze down more than 10 vials of cells for future use at early passage.

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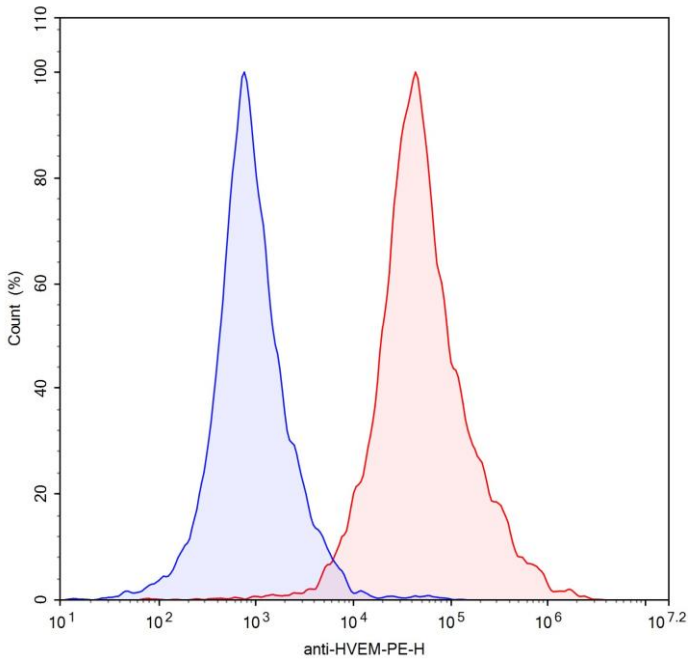
Validation

Cell surface expression of human HVEM in HVEM-HEK293 cells was confirmed by flow cytometry.

Figure 1. Flow cytometry analysis of cell surface expression of HVEM in HVEM-HEK293 cells.

HVEM-HEK293 cells (red) or control HEK293 cells (blue) were stained with PE-labeled Anti-HVEM Antibody (Biolegend, #318806) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE.



	Samples	Subset	Cell Count
	Control HEK293 Cell	Live Singlet	7,642
	HVEM-HEK293 Cell	Live Singlet	2,917

Sequence

HVEM sequence (Genbank accession number NM_003820)

MEPPGDWGPPPWRSTPKTDVLRLVLYLTFLGAPCYAPALPSCKEDEYPVGSECCPKC
SPGYRVKEACGELTGTVCEPCPPGTYIAHLNGLSKCLQCQMCDPAMGLRASRNCSRT
ENAVCGCSPGHFCIVQDGDHCAACRAYATSSPGQRVQKGGTESQDTLCQNCPPGTF
SPNGTLEECQHQTKCSWLVTKAGAGTSSSHWVWWFLSGSLVIVIVCSTVGLIICVKRR
KPRGDVVKVIVSVQRKRQEAEGEATVIEALQAPPDVTTVAVEETIPSFTGRSPNH

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Related Products

Product	Cat. #	Size
LIGHT-CHO Recombinant Cell Line	79262	2 vials
LIGHT, His-Tag (Mouse)	79068	100 µg
NF-kB Reporter Kit	60614	500 reactions
HVEM CHO Recombinant Cell Line	79297	2 vials
HVEM, Fc Fusion (Human)	71142	100 ug
HVEM, Fc fusion, Biotin-labeled (Human)	71143	50 µg
Thaw Medium 1	60187	100 ml
Thaw Medium 3	60186	100 ml
BTLA:HVEM [Biotinylated] Inhibitor Screening Assay Kit	72008	96 reactions
BTLA: HVEM Screening & Profiling		

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