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## **Data Sheet**

### **HVEM - CHO Recombinant Cell Line**

#### **Cat #: 79297**

#### **Product Description**

Recombinant CHO-K1 stably expressing human HVEM (TNF receptor superfamily member 14; TNFRSF14; TR2; ATAR; HVEA; HVEM; CD270; LIGHTR; 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 expressed on the surface of T cells. Binding of HVEM to one of its ligands, LIGHT (CD258, TNFSF14) or LT $\alpha$  (lymphotoxin- $\alpha$ ), causes a co-stimulatory signal which can activate lymphoid cells. 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

#### **Format**

Each vial contains  $\sim 2 \times 10^6$  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 3 (BPS Cat. #60186):** Ham's F-12 medium (Hyclone, #SH30526.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

**Growth Medium 3E (BPS Bioscience #79553):** Thaw Medium 3 (BPS Cat. #60186) plus 100  $\mu$ g/ml of Hygromycin B (Life Technologies #10687-010)

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Cells should be grown at 37°C with 5% CO<sub>2</sub> using Growth Medium 3E to ensure recombinant expression. HVEM CHO cells should display a typical cell division time of about ~17 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 3 (**no Hygromycin B**), spin down cells at 1000 rpm and resuspend cells in 5 ml of pre-warmed Thaw Medium 3 (**no Hygromycin**). Transfer resuspended cells to T25 flask and culture at 37°C in a 5% CO<sub>2</sub> incubator overnight. The next day, replace the medium with fresh warm Thaw Medium 3 (**no Hygromycin B**), and continue growing culture in a CO<sub>2</sub> 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 3E (**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 3E (**contains Hygromycin B**) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 3E (**contains Hygromycin B**) and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ratio: 1:10 to 1:20 weekly or twice a week.

**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 3 (**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 ~2x10<sup>6</sup> 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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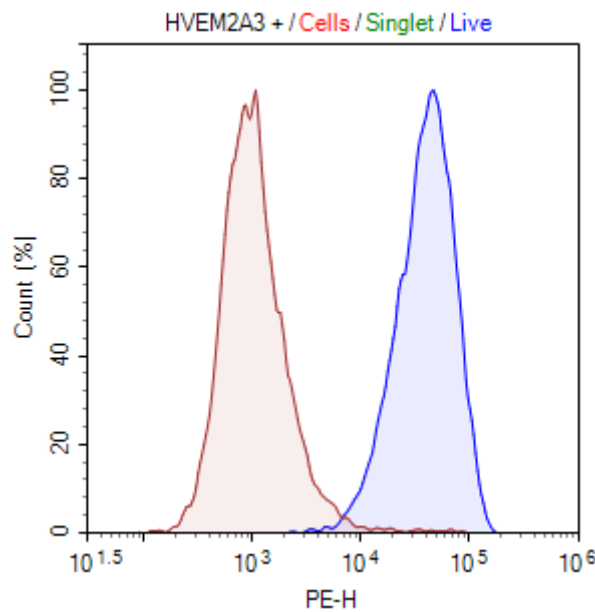
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**Validation**

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

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

HVEM-CHO cells (blue) or control CHO cells (red) 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	Mean X
	HVEM-CHO Cell	Live Singlet	5,792	47,104
	Control CHO Cell	Live Singlet	9,020	1,677

**Sequence**

HVEM sequence (accession number NM\_003820)

```
MEPPGDWGWPPWRSTPKTDVLRRLVLYLTFLGAPCYAPALPSCKEDEYPVGSECCPKCSPGYRVKE
ACGELTGTVCEPCPPGTYYIAHLNGLSKLQCMCDPAMGLRASRNCNCRTEHAVCGCSPGHFCIVQ
DGDHCAACRAYATSSPGQRVQKGGTESQDTLCQNCPPGTFSNGLTEECQHQTCSWLVTKAGAG
TSSSHVWVWFLSGSLVIVIVCSTVGLIICVKRRKPRGDVVKVIVSVQRKRQEAEGEATVIEALQA
PPDVTTVAVEETIPSFTRSPNH
```

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## Related Products

<b><u>Product</u></b>	<b><u>Cat. #</u></b>	<b><u>Size</u></b>
LIGHT-CHO Recombinant Cell Line	79262	2 vials
NFAT Reporter (Luc) – Jurkat Recombinant Cell Line	60621	2 vials
NF-kB reporter (Luc) - Jurkat Recombinant Cell line	60651	2 vials
NF-kB Reporter Kit	60614	500 reactions
HVEM, Fc fusion (Human)	71142	100 µg
HVEM, Fc fusion, Biotin-labeled (Human)	71143	50 µg
LIGHT, His-Tag (Mouse)	79068	100 µg
BTLA:HVEM [Biotinylated] Inhibitor Screening Assay Kit	72008	96 reactions
Thaw Medium 3	60186	100 ml
BTLA: HVEM Screening & Profiling		

## Notes

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