

Mouse Recombinant HVEM Stable Cell Line
Cat. No. M00647**Version 07072017****I. INTRODUCTION**

Catalog Number: M00647

Cell Line Name: CHO-K1/mouse HVEM

Gene Synonyms: TNFRSF14

Expressed Gene: Codon Optimized from NM_178931.2;no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (1×10^6 per vial)

Stability: 15 passages

Application: Binding assay or use as immunogen

Freeze Medium: 95% complete growth medium, 5% DMSO

Complete Growth Medium: F12K, 10% FBS

Culture Medium: F12K, 10% FBS, 8 μ g/ml PuromycinMycoplasma Status[§]: Negative

Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

HVEM is found on the surface of various cell types, including hematopoietic and non-hematopoietic cells, and is expressed mainly in spleen, thymus, bone marrow, lung and intestines. It has been identified as a canonical TNF receptor, signaling through the TNF receptor-associated factors (TRAFs) leading to NF κ B activation. Furthermore, in addition to acting as a signaling receptor, in binding to Ig superfamily members it acts as a ligand for these inhibitory receptors. Therefore, bidirectional signaling is possible for the HVEM-mediated signaling network, which can be involved in positive or negative immunological reactions under different contexts.

[§]: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of *M. fermentans*, *M. hyorhinis*, *M. arginini*, *M. orale*, *M. salivarium*, *M. hominis*, *M. pulmonis*, *M. arthritis*, *M. neurolyticum*, *M. hyopneumoniae* and *M. capricolum*) and one species *Ureaplasma* (*U. urealyticum*), with sufficient sensitivity and specificity.

III. REPRESENTATIVE DATA

Protein Expression Validation

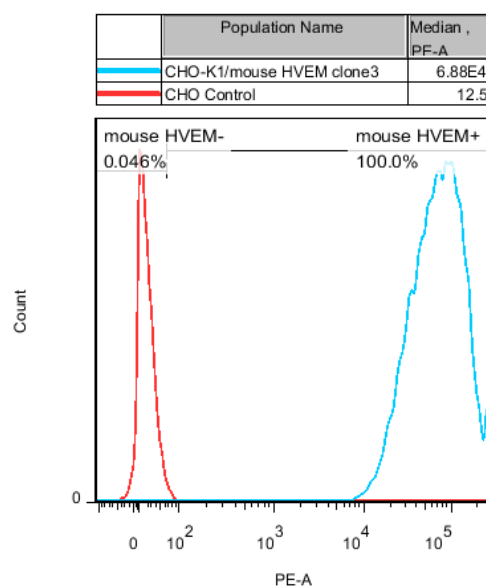


Figure 1. FACS analysis of mouse HVEM expression in CHO-K1 cells.

IV. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 200 x g for 5 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5 % CO₂.
7. Add antibiotic the following day.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.25% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25200-072) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g for 5 min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5% CO₂.

Subcultivation Ratio: 1:4 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Murphy TL, Murphy KM. Slow down and survive: Enigmatic immunoregulation by BTLA and HVEM [J]. Annu Rev Immunol, 2010, 28: 389–411.
2. Hsu H, et al. A novel tumor necrosis factor receptor family member, signals through TRAF2 and TRAF5 [J]. J Biol Chem, 1997, 272:13471–13474.
3. Steinberg MW, et al. Regulating the mucosal immune system: the contrasting roles of LIGHT, HVEM, and their various partners [J]. Semin Immunopathol, 2009, 1: 207–221.

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