

Cyno Recombinant VISTA Stable Cell Line
Cat. No. M00634**Version 06062017****I. INTRODUCTION**

Catalog Number: M00634

Cell Line Name: CHO-K1/cyno VISTA

Gene Synonyms: C9orf54; C9H10orf54

Expressed Gene: Codon Optimized from NM_001190878; 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

V-domain Ig suppressor of T cell activation (VISTA) is a potent negative regulator of T-cell function that is expressed on hematopoietic cells. VISTA levels are heightened within the tumor microenvironment, in which its blockade can enhance antitumor immune responses in mice. In humans, blockade of the related programmed cell death 1 (PD-1) pathway has shown great potential in clinical immunotherapy trials.

[§]: 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. arthritidis*, *M. neurolyticum*, *M. hyopneumoniae* and *M. capricolum*) and one species *Ureaplasma* (*U. urealyticum*), with sufficient sensitivity and specificity.

III. REPRESENTATIVE DATA

- Protein Expression Validation

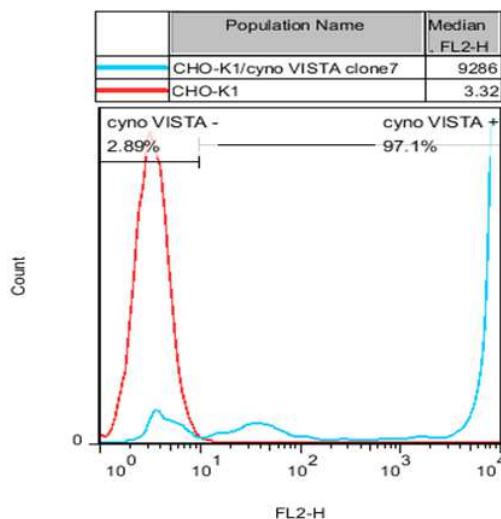


Figure 1. FACS analysis of cytochrome VISTA expression in CHO-K1 cells.

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. Mahoney KM1, Rennert PD2, Freeman GJ3. Combination cancer immunotherapy and new immunomodulatory targets [J]. Nat Rev Drug Discov. 2015 Jul 31; 14(8): 561-84.
2. Lines JL1, Pantazi E, Mak J, Sempere LF, Wang L, O'Connell S, Ceeraz S, Suriawinata AA, Yan S, Ernstoff MS, Noelle R. VISTA is an immune checkpoint molecule for human T cells [J]. Cancer Res. 2014 Apr 1; 74(7):1924-32.

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