Human Recombinant CD16A 158F Stable Cell Line
Cat. No. M00586 **Version 04282015**

I. INTRODUCTION

Catalog Number: M00586
Cell Line Name: CHO-K1/CD16A 158F
Gene Synonyms: FCGR3A, FCG3, FCGR3
Expressed Gene: Codon Optimized from NM_000569.6; 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: F-12K, 10% FBS
Culture Medium: F-12K, 10% FBS, 100 μg/ml Hygromycin B, 4 μg/ml Puromycin
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

CD16 is a cluster of differentiation molecule found on the surface of natural killer (NK) cells, monocytes and macrophages. It can be used to isolate populations of NK cells by antibodies directed towards CD16, using fluorescent-activated cell sorting or magnetic-activated cell sorting. CD16 has been identified as Fc receptors FcγRIIIa (CD16a) and FcγRIIIb (CD16b). The two isoforms of the CD16a stimulatory receptor found on NK cells and macrophages differ by one amino acid at codon 158 (V/F) and are important for antibody-dependent cell-mediated cytotoxicity (ADCC). The high affinity 158 V/V homodimer of CD16A is present on approximately 20% of the population and the lower affinity F/F homodimer and F/V heterodimer are present in the remaining 80%.

§: 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

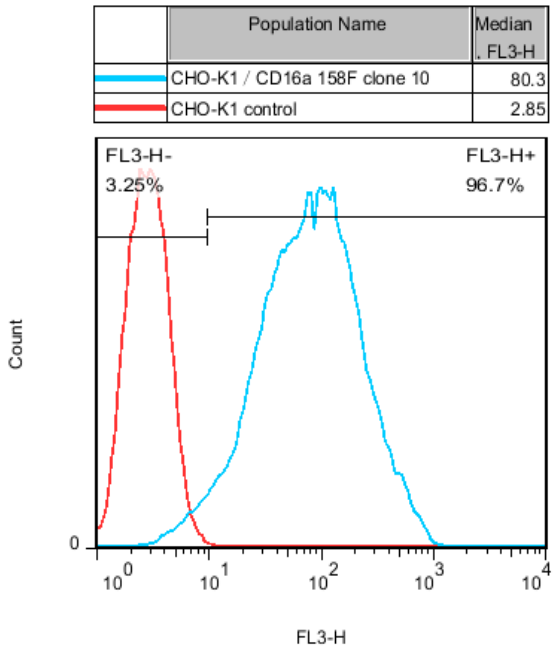

Figure 1. Flow cytometry analysis of CD16A 158F protein expression in CHO-K1/CD16A 158F cells.

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 %CO_2.
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 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 1 to 2 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 5min, 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 %CO_2.

Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Janeway, Charles (2001). "Appendix II. CD antigens". Immunobiology (5ed.). New York: Garland. ISBN 0-8153-3642-X.
2. Moraru M, Black LE, Muntasell A, Portero F, López-Botet M, Reyburn HT, Pandey JP, Vilches C. "NK Cell and Ig Interplay in Defense against Herpes Simplex Virus Type 1: Epistatic Interaction of CD16A and IgG1 Allotypes of Variable Affinities Modulates Antibody-Dependent Cellular Cytotoxicity and Susceptibility to Clinical Reactivation". J Immunol. 2015 Aug 15; 95 (4):1676-84.
3. Lehrnbecher T, Foster CB et al. Blood. 1999;94:4220–32

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