**Human Recombinant CD64 Stable Cell Line**
**Cat. No. M00588** **Version 05162017**

## I. INTRODUCTION

Catalog Number: M00588
Cell Line Name: CHO-K1/CD64
Gene Synonyms: CD64; FCRI; CD64A; IGFR1
Expressed Gene: Codon Optimized from NM_000566.3; NM_004106.1; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells ($1\times10^6$ 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, 300μg/ml HygromycinB, 8 μg/ml Puromycin.
Mycoplasma Status[§]: Negative
Storage: Liquid nitrogen immediately upon receipt

## II. BACKGROUND

CD64 (Cluster of Differentiation 64) is a type of integral membrane glycoprotein known as an Fc receptor that binds monomeric IgG-type antibodies with high affinity. It is more commonly known as Fc-gamma receptor 1 (FcγRI). After binding IgG, CD64 interacts with an accessory chain known as the common γ chain (γ chain), which possesses an ITAM motif that is necessary for triggering cellular activation. CD64 is constitutively found on only macrophages and monocytes, but treatment of polymorphonuclear leukocytes with cytokines like IFNγ and G-CSF can induce CD64 expression on these cells.

*§: 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

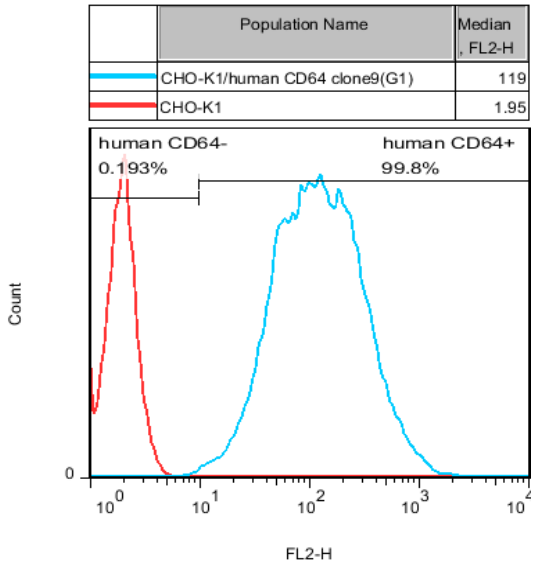


**Figure 1.** FACS analysis of human CD64 expression in CHO-K1 cells.

## 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 % $CO_2$.
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 0.25% (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% $CO_2$.

Subcultivation Ratio: 1:4 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

## V. REFERENCES

1. Hulett M, Hogarth P. The second and third extracellular domains of FcgammaRI (CD64) confer the unique high affinity binding of IgG2a [J]. Mol Immunol.1998, 35 (14-15): 989–996.
2. Nimmerjahn F, Ravetch J. Fcgamma receptors: old friends and new family members [J]. Immunity, 2006, 24 (1): 19–28.
3. Perussia B, Dayton E, Lazarus R, Fanning V, Trinchieri G. Immune interferon induces the receptor for monomeric IgG1 on human monocytic and myeloid cells [J]. J Exp Med, 1983, 158 (4): 1092–1113.
4. Repp R, et al. Neutrophils express the high affinity receptor for IgG (Fc gamma RI, CD64) after in vivo application of recombinant human granulocyte colony-stimulating factor [J]. Blood, 1991, 78 (4): 885–889.

**GenScript USA Inc**,
860 Centennial Ave.
Piscataway, NJ 08854
Toll-Free: 1-877-436-7274
Tel: 1-732-885-9188, Fax: 1-732-210-0262
Email: product@genscript.com
Web: http://www.genscript.com
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