Human Recombinant CD32A 131 His Stable Cell Line
Cat. No. M00598 **Version 04282015**

I. INTRODUCTION

Catalog Number: M00598
Cell Line Name: CHO-K1/human CD32A 131 His
Gene Synonyms: CD32; FCG2; FcGR; CD32A; CDw32; FCGR2; IGFR2; FCGR2A1
Expressed Gene: Codon Optimized from NM_001136219.1; 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 μg/ml Puromycin
Mycoplasma Status[§]: Negative
Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

CD32 is a surface receptor protein and part of a large population of B cell co-receptors, which act to modulate signaling. It has a low-affinity for IgG antibodies and down-regulates antibody production in the presence of IgG. This feedback loop acts to lower the production of IgG by B cells when there is a surplus in the body. Monoclonal antibodies can distinguish between CD32A and CD32B.

§: 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

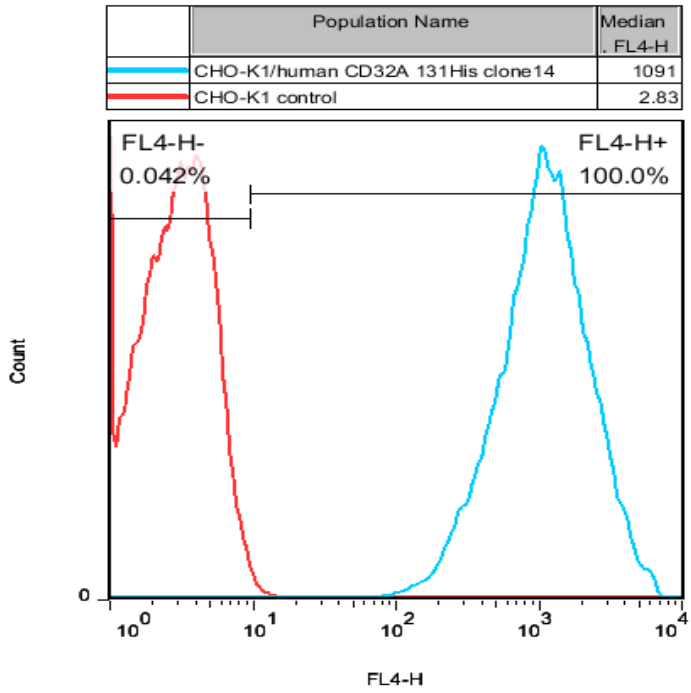

Figure 1. FACS analysis of human CD32A 131 His expression in CHO-K1 cells.

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

V. REFERENCES

1. Sondermann, P., Huber, R., Jacob, U. Crystal structure of the soluble form of the human fcgamma-receptor IIb: a new member of the immunoglobulin superfamily at 1.7 A resolution [J]. EMBO, 1999, 18 (5): 1095–1103.

2. Veri, M.C., Gorlatov, S., Li, H., et al. (2007). Monoclonal antibodies capable of discriminating the human inhibitory Fcgamma-receptor IIB (CD32B) from the activating Fcgamma-receptor IIA (CD32A): biochemical, biological and functional characterization [J]. Immunology. 121 (3): 392–404.

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