Human Recombinant CD32A 131Arg Stable Cell Line
Cat. No. M00887

Version 01

Update 03292022

I. INTRODUCTION

Recombinant CHO-K1 cells stably overexpress immunoglobulin gamma Fc region receptor II-a (FcγRIIa / CD32A 131Arg). The surface expression of CD32A 131Arg is validated by FACS analysis. This stable cell line product is designed for measuring binding affinity and stability of antibody based biologics binding with CD32A 131Arg. GenScript offers both CD32A 131Arg stable cell line and CD32A 131His stable cell line (Cat. No. M00589) for FcγRIIa polymorphism study.

Catalog Number: M00887
Cell Line Name: CHO-K1/human CD32A 131Arg
Gene Synonyms: CD32; FCG2; FcGR; CD32A; CDw32; FCGR2; IGFR2; FCGR2A1
Expressed Gene: Codon optimized from NM_001136219.1; no expressed tags
Target Protein: NP_001129691.1
Host Cell: CHO-K1
Quantity: 2 vials of frozen cells (>1X10^6 per vial in 1 ml)
Culture Properties: Adherent
Freeze Medium: 95% complete growth medium, 5% DMSO (Cat. No. D2650, Sigma)
Complete Growth Medium: F-12K (Cat. No. 21127-022, Gibco), 10% FBS (Cat. No. 10099-141, Gibco)
Culture Medium: F-12K, 10% FBS, 8 μg/ml Puromycin (Cat. No. A11138-03, Gibco)
Stability: Stable through more than 15 passages with no significant changes in assay performance or expression profile.
Applications: Cell-based binding assay; use as immunogen for antibody development
Mycoplasma Status: Negative. The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit (Cat. No. LT07-318, Lonza).
Storage: Store cells in liquid nitrogen immediately upon receipt. Thaw and recover cells within one year from the date received.

II. BACKGROUND

Human CD32 group consists of FcγRIIA, B and C proteins that share 94-99% amino acid identity in their extracellular domains but differ substantially in their transmembrane and cytoplasmic domains. CD32A, also known as FcγRIIA, is a surface receptor protein and part of a large population of B cell co-receptors. The protein is found on phagocytic cells such as macrophages and neutrophils, and it is involved in the process of phagocytosis and clearing of immune complexes. It has 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. Therefore, targeting CD32A may represent an approach to immunotherapy of B-cell malignancies.

III. REPRESENTATIVE DATA

FACS Analysis

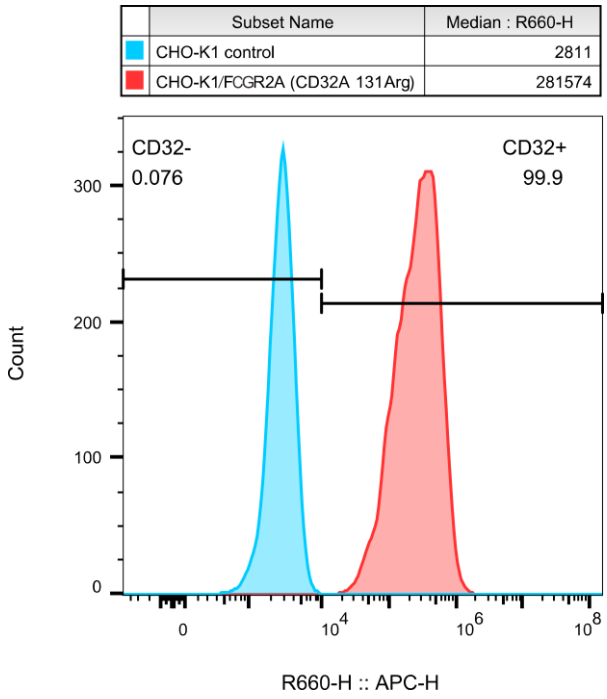

Figure 1. FACS analysis of cell surface expression of CD32A 131Arg on CHO-K1 cells. CHO-K1/CD32A 131Arg cells (red) and negative control CHO-K1 cells (blue) were probed using APC Mouse Anti-Human CD32 antibody (Cat. No. 303208, Biolegend).

Stability Validation

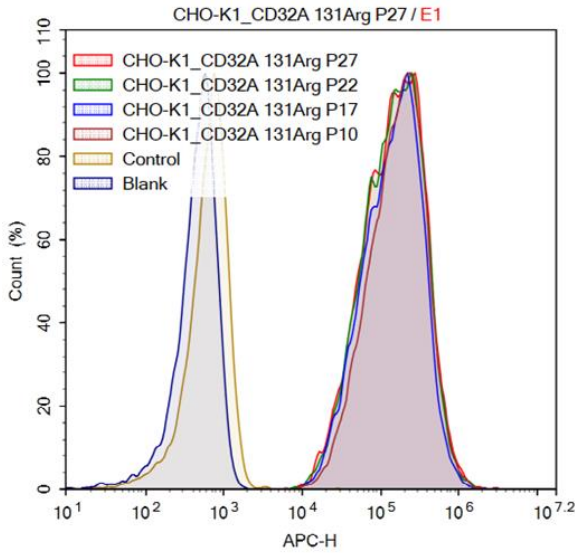

Figure 2: FACS analysis of cell surface expression of CD32A 131Arg on CHO-K1 cells from different passages. CHO-K1/CD32A 131Arg cells and negative control CHO-K1 cells were probed using APC Mouse Anti-Human CD32 antibody (Cat. No. 559769, BD Biosciences). P10, P17, P22, and P27 represent cell passage numbers.

IV. THAWING, SUBCULTURING AND CRYOPRESERVATION

Thawing Protocol

1. Remove the vial containing the frozen cells from liquid nitrogen tank and place into a 37°C water bath immediately.
2. Thaw the cells quickly (within 1-2 minutes) by gently swirling the vial. Do not vortex the cells.
3. When the cells are almost completely thawed, take the vial out of the water bath and decontaminate it with 70% ethanol.
4. In a biosafety hood, transfer the cells to a sterile 15 ml conical tube. Add 9 ml of complete growth medium to the cells.
5. Pellet cells by centrifugation at 200 × g for 3-5 minutes at room temperature.
6. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure that the cell pellet is not disturbed.
7. Resuspend the cells by gently flicking the tube. Gently add in 10 ml of complete growth medium.
8. Transfer the cell suspension into a 10 cm culture dish containing 10 ml of complete growth medium.
9. Grow the cells in an incubator at 37°C with 5% CO_2.

Sub-culturing Protocol

For CHO-K1, HEK293, HEK293T, HT1080 or other adherent cell lines:

1. Remove the culture medium from the cells.
2. Wash cells with sterile PBS to remove all traces of serum which contains trypsin inhibitors.

3. Add Trypsin/EDTA solution to the culture dish and observe the cells under an inverted microscope until the cell layer has dispersed (usually within 3-5 minutes).
   Notes: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. If cells are difficult to detach, place the dish in a 37°C incubator for about 2 minutes.
4. Add 6-8 ml of complete growth medium to the culture dish, aspirate the medium with cells by gentle pipetting and then add into a sterile falcon tube.
5. Centrifuge the cells at 200 x g for 5 minutes, and remove the medium.
6. Resuspend the cells in culture medium and add the cell suspension to a new culture dish.
7. Grow the cells in an incubator at 37°C with 5% CO_2.
   Notes:
   Subcultivation Ratio: 1:3 to 1:20
   Medium Renewal: Every 2 to 3 days.

For Jurkat or Daudi or other suspension cell lines:

1. Centrifuge the cells at 200 x g for 5 minutes, and remove the medium.
2. Resuspend the cells in culture medium and add the cell suspension to a new culture dish.
3. Grow the cells in an incubator at 37°C with 5 % CO_2.
   Notes:
   Subcultivation density: 2.5 ×10^5 cells/ml ~ 4 ×10^5 cells/ml.
   Medium Renewal: Every 2 to 3 days.

Cryopreservation Protocol

*For CHO-K1, HEK293, HEK293T, HT1080 or other adherent cell lines, start at **Step 1**.*

*For Jurkat or Daudi or other suspension cell lines, start at **Step 3.***

1. Remove the cell culture medium, wash the cells with PBS once (optional), gently add enough trypsin to cover the cells and incubate for approximately 2 minutes in a 37°C incubator.
2. Resuspend in cell culture medium and transfer into a sterile 50 ml conical tube.
3. Count the viable cells using a hemocytometer. If preferred, also determine the cell viability. Cell viability should be at least 90% for good cryopreservation.
4. Centrifuge the cells at about 200 × g for 5 minutes at room temperature to pellet cells. Remove the supernatant gently without disturbing the cell pellet.
5. Resuspend cells by adding freezing medium to the tube to the required cell density (2-5 × 10^6 cells/ml for best results).
6. Aliquot 1 ml each into cryogenic storage vials and secure the lids.
7. Transfer the vials into a cryo-freezing container at room temperature and put into a -80°C freezer. The temperature inside the cryo-freezing container should decrease steadily by 1°C/minute.
8. After approximately 24 hours, remove the vials from the cyro-freezing container and transfer into liquid nitrogen for long term storage.

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. 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. 2007, 121 (3): 392–404.
3. Herkenham M et al. Cannabinoid receptor localization in brain. Proc Natl Acad Sci U S A. 1990, 87(5):1932-6.

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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
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