Certificate of Analysis

Product Cat. No.: M00578

Host Cell: CHO-K1

Target gene: Cyno Flt3

Shipping Conditions: Dry ice

Lot Number: B30051702

860 Centennial Ave., Piscataway, NJ 08854, USA
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Stable Cell Line Information

Recommended Cell Culture Medium: F-12K (Gibco, Cat. #21127-022), FBS (Gibco, Cat. #10099-141), and 6μg/ml Puromycin* (Gibco, Cat. #10131-027)

Freeze Medium: 95% complete growth medium, 5% (V/V) DMSO

Description: One stable subline using CHO-K1 as the host will be established to overexpress Cyno Flt3

QC: FACS

Mycoplasma 160 Test:** Negative

** Concentration used for selection was 6 μg/ml Puromycin.*

*** Our PCR mycoplasma test covers 11 of the most common species of mycoplasma, with sufficient sensitivity and specificity.*

Notice to Purchaser:

GenScript stable cell line products are to be used for research purposes only, not intended for use in humans. GenScript products may not be transferred to third parties or used to manufacture commercial products without written approval. Use of this product is also subject to compliance with the licensing requirements.

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QC Data

1. Validation by Flow Cytometry

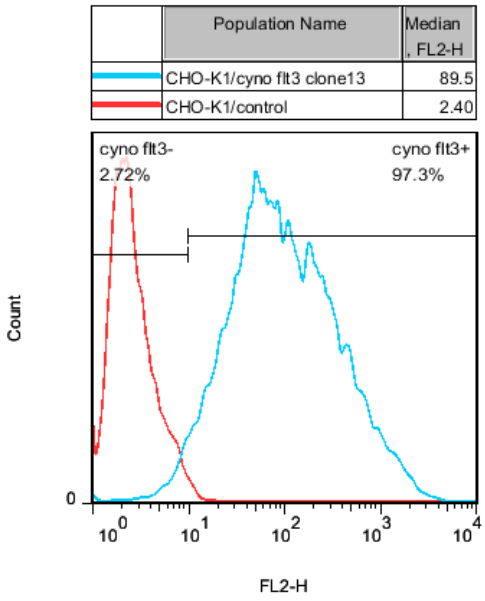

Figure 1. FACS analysis of Cyno Flt3 in CHO-K1/ Cyno Flt3

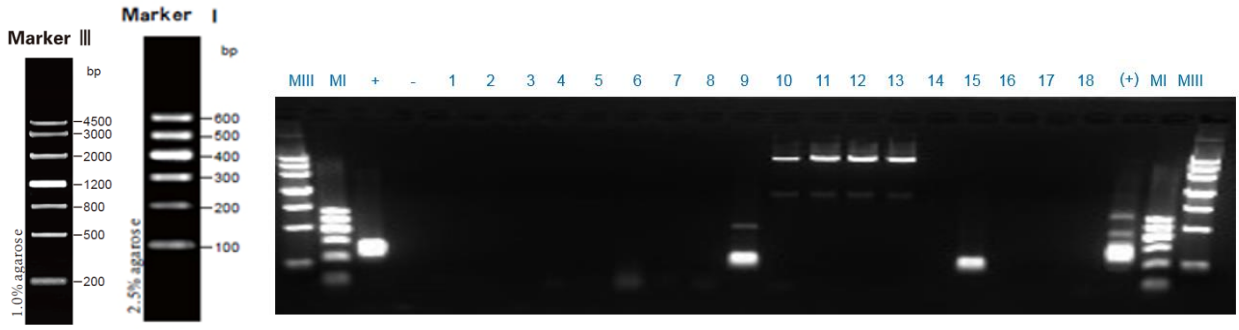

Figure 2. Myco 160 analysis of Cyno Flt3 in CHO-K1/ Cyno Flt3 (Lane 16: M00578)

Instruction for maintaining stable cell line

Cell recovery

The cells were maintained in F-12K, 10% FBS, and 6 μg/ml Puromycin. The S.O.P for cell recovery is briefly introduced here:

1) Prepare a 37°C water bath.

2) Take the cryovial out of the liquid nitrogen tank and thaw it by gentle swirling in the water bath until ice crystals are melted, usually within 1-3 minutes.

3) Decontaminate the vial by spraying with 70% ethanol.

4) Unscrew the vial and transfer the cells into a 15 ml sterile conical centrifuge tube containing 9 ml pre-warmed complete growth medium.

5) Centrifuge the tube at 125 g for 10 minutes, discard the supernatant and resuspend the cells in 2 ml complete growth medium. Pipette gently to loosen the pellet.

6) Transfer the cell suspension to the culture vessel with antibiotic free medium and mix well. Incubate the vessel at 37°C, 5% CO_2.

7) Replace the medium with fresh culture medium the next day (with appropriate concentration of antibiotic).

Cell Maintenance and Subculturing

Volumes listed below are for 10 cm dish, proportionally reduce or increase the volume for culture vessels of other sizes.

1) Balance the complete growth medium to 37°C in a water bath.

2) Discard the culture medium of the dish.

3) Briefly rinse the cell layer with Ca^{2+}/Mg^{2+} free DPBS to remove all traces of serum.

4) Add 1.0 to 2.0 ml of Trypsin-EDTA solution to the dish and observe cells under an inverted microscope until cell layer is dispersed (usually within 1 to 3minutes).

Note: To avoid clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate the dispersal.

5) Add 8.0 ml of complete growth medium and suspend the cells by gently pipetting.

6) Centrifuge the cells at 125 g for 10 minutes, discard the supernatant and resuspend the cells in 5 ml complete growth medium.

7) Add appropriate aliquot of the cell suspension to new culture vessel.

Subcultivation Ratio: 1:3 to 1:6

Medium Renewal: 2-3 times per week

Cell Cryopreservation

1) Prepare a freeze medium consisting of complete growth medium and 5% DMSO.

2) Harvest cells by gentle centrifugation at 125 g for 10 minutes and resuspend them in the freeze medium at a concentration of 1 x 10^6 to 5 x 10^6 viable cells/ml. Rest of the cells are cultured until the viability of the recovered cells is confirmed.

3) Label the cryovials with the name of the cell line, then add 1 ml of the cell suspension to each of the vials.

4) Place the vials into a pre-cooled (4°C) controlled-rate freeze chamber and place the chamber in a -80°C freezer for at least 24 hours.

5) Quickly transfer the vials to a liquid nitrogen tank.

6) After 24 hours' preservation in liquid nitrogen, take one vial out and culture the cells to check the cell viability.

Packing List of M00578

Cell lines (Shipping Condition: -80°C Dry Ice, Store at -196°C)

Name: CHO-K1/ Cyno Flt3
Quantity: 1x10^6cells/vial
Lot No.: Cyno Flt3
Number of vial: 2 vials
Store at: -196°C

Certified by: Jan sun **Date:** ***01/17/2018***

Appendix

1. Target gene information

Cyno Flt3, XM_001117913.2.;

2. Antibodies used for FACS

Primary antibody: PE anti-human CD135 (Flt-3/Flk-2) (Biolegend,Cat#313306)

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