Human Recombinant Gα15 Stable Cell Line

Technical Manual No. TM0609 **Version 11012010**

I. Introduction

Catalog Number: M00257

Cell Line Name: CHO-K1/Gα15

Gene Synonyms: GNA15, GNA16

Official Full Name: Guanine nucleotide binding protein (G protein), alpha 15 (Gq class)

Expressed Gene: GenBank Accession Number NM_002068; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (3×10^6 per vial)

Application: Functional assay for Gs and Gi/o-coupled GPCR receptors

Freeze Medium: 45% complete growth medium, 45% FBS, 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

1. CHO-K1/Gα15

CHO-K1/Gα15 is a CHO-K1 cell line stably expressing the Gα15 alpha subunit protein which a Gq protein. It is used as a host cell for transfection expression of Gs and Gi/o -coupled receptors, the constitutively expressed Gα15 protein in the cells allows many transfected receptors which normally stimulate/inhibit the cAMP pathway, to couple to Gq signal transduction and mobilize intracellular calcium. The cell line carries the hygromycin B resistance gene and is resistant to hygromycin B.

2. The sequence of Gα15

ATGGCCCGCTCGCTGACCTGGCGCTGCTGCCCCTGGTGCCTGACGGAGGATGAGAAGGCCGCCGCCCGGG
TGGACCAGGAGATCAACAGGATCCTCTTGGAGCAGAAGAAGCAGGACCGCGGGGAGCTGAAGCTGCTGCT
TTTGGGCCCAGGCGAGAGCGGGAAGAGCACCTTCATCAAGCAGATGCGGATCATCCACGGCGCCGGCTAC
TCGGAGGAGGAGCGCAAGGGCTTCCGGCCCCTGGTCTACCAGAACATCTTCGTGTCCATGCGGGCCATGA
TCGAGGCCATGGAGCGGCTGCAGATTCCATTCAGCAGGCCCGAGAGCAAGCACCACGCTAGCCTGGTCAT
GAGCCAGGACCCCTATAAAGTGACCACGTTTGAGAAGCGCTACGCTGCGGCCATGCAGTGGCTGTGGAGG
GATGCCGGCATCCGGGCCTACTATGAGCGTCGGCGGGAATTCCACCTGCTCGATTCAGCCGTGTACTACC

§: 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

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TGTCCCACCTGGAGCGCATCACCGAGGAGGGCTACGTCCCCACAGCTCAGGACGTGCTCCGCAGCCGCAT
GCCCACCACTGGCATCAACGAGTACTGCTTCTCCGTGCAGAAAACCAACCTGCGGATCGTGGACGTCGGG
GGCCAGAAGTCAGAGCGTAAGAAATGGATCCATTGTTTCGAGAACGTGATCGCCCTCATCTACCTGGCCT
CACTGAGTGAATACGACCAGTGCCTGGAGGAGAACAACCAGGAGAACCGCATGAAGGAGAGCCTCGCATT
GTTTGGGACTATCCTGGAACTACCCTGGTTCAAAAGCACATCCGTCATCCTCTTTCTCAACAAAACCGAC
ATCCTGGAGGAGAAAATCCCCACCTCCCACCTGGCTACCTATTTCCCCAGTTTCCAGGGCCCTAAGCAGG
ATGCTGAGGCAGCCAAGAGGTTCATCCTGGACATGTACACGAGGATGTACACCGGGTGCGTGGACGGCCC
CGAGGGCAGCAAGAAGGGCGCACGATCCCGACGCCTCTTCAGCCACTACACATGTGCCACAGACACACAG
AACATCCGCAAGGTCTTCAAGGACGTGCGGGACTCGGTGCTCGCCCGCTACCTGGACGAGATCAACCTGC
TGTGA

III. 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 force for 5 min, and discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add Hygromycin B to a concentration of 100 μg/ml in the following day.

Subculturing protocol

1. Remove and discard culture medium.
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 to 10 cm dish and observe the cells under an inverted microscope until cell layer is detached (usually within 3 to 5 minutes).
   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 detaching.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in the culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

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

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