MANUAL

GFP Stably expressing CHO Cell Line

Catalog #: GFP-0001

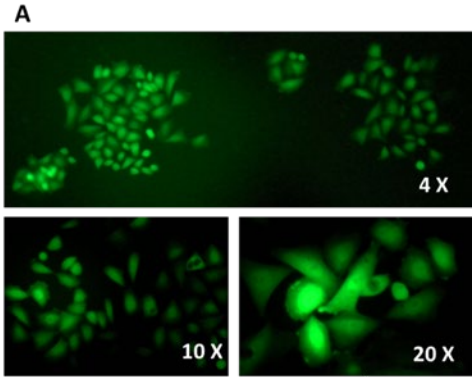
ISO 13485

Introduction

The CHO cell line is an adherent epithelial cell derived from the ovary of Chinese hamster. It is widely used in biology and medical research of genetics, toxicity screening, nutrition, and gene expression, particularly to express recombinant proteins. CHO cells are the most common mammalian hosts for industrial production of recombinant protein therapeutics.

CHO-GFP stable cell line is transformed from the CHO cell line and stably expresses the GFP fluorescent protein. Both GFP and geneticin-resistant genes are introduced into parental CHO cells using retrovirus. Strong Green fluorescent signal can be visualized in every single cell under fluorescent microscope. The expression of GFP has been validated by western blot.

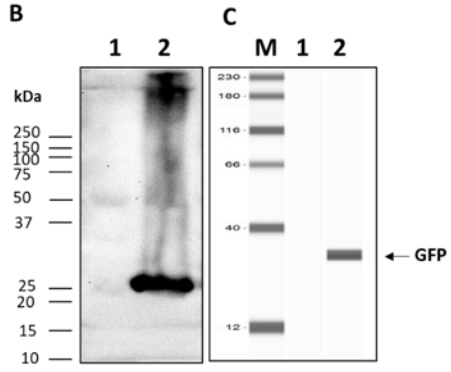
Protein Accession Number: P42212.



A. Fluorescence microscope images.

B. Western blot analysis.

C. Simple western analysis.



Lane M: Protein marker.

1: CHO

2: CHO/GFP

Rabbit anti-GFP: 130-11011 (1:1000)

Provided Materials

One vial of 2 x 10^6 cells, at P4 in Freezing Media.
IMPORTANT: store the frozen cells in liquid nitrogen until you are ready to thaw and propagate them.

Additional Materials Required

1. Minimum Essential Medium α (MEM α)
2. Fetal Bovine Serum (FBS)
3. Penicillin/Streptomycin
4. Trypsin
5. Phosphate-buffered saline (PBS)
6. DMSO
7. 96-well white plate

Handling Cells Upon Arrival

It is strongly recommended that you propagate the cells following instructions as soon as possible upon arrival. **IMPORTANT**: An adequate number of frozen stocks must be made from early passages as cells will undergo genotypic changes. Genetic instability in transfected cells will result in a decreased responsiveness over time in normal cell culture conditions.

Required Cell Culture Media

Complete Growth Media

In 450mL of MEM α, add 50mL FBS (10% final) and 5mL Penicillin/Streptomycin (1% final).

Freezing Media

Add 10% DMSO (final) to Complete Growth Media and sterile filter. Make fresh each time.

Initial Culture Procedure

1. Quickly thaw cells in a 37 °C water bath with careful agitation. Remove from the bath as soon as the vial is thawed.
2. Transfer cells to a 15ml centrifuge tube containing 7ml of pre-warmed Complete Growth Media.
3. Centrifuge tube at 1200-1500 RPM for 5 minutes.
4. Remove supernatant and resuspend cells with 1ml Complete Growth Media.
5. Transfer cells to a T75cm^2 tissue culture flask or 100 mm culture dish containing 8-12ml of Complete Growth Media.
6. Place the flask with cells in a humidified incubator at 37 °C with 5% CO_2

Subculture Procedure

A sub-cultivation ratio of 1:3 to 1:4 is recommended with media changes every 2 to 3 days.

Preparing Frozen Stocks

This procedure is designed for 60mm^2 dish or T25cm^2 flask. Scale volumes according to other vessels.

1. When cells reach 1-1.5x10^6/ml, freeze down cells.
2. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 1200-1500 RPM for 5 minutes to collect the cells into a pellet.
3. Carefully aspirate the media and resuspend cells in 1ml freezing media and gently resuspend by pipetting up and down.
4. Transfer 1mL of cells into a cryogenic vial.
5. Place the cryogenic vial in a freezing container and store it at -80 °C freezer overnight.

6. Transfer cells to liquid nitrogen for long-term storage.

This product is for research use only.