Signosis
Innovative Plate Assay Solutions

Epidermal Growth Factor Receptor (EGFR) Stable Cell Line

(For Research Use Only)

EGFR (E19del) Stably Expressing HCC827 Stable Cell Line – Catalog Number: EL-005
EGFR (E19del+T790M) Stably Expressing HCC827 Stable Cell Line – Catalog Number: EL-006
Control (Empty Vector) HCC827 Stable Cell Line - Catalog Number: EL-007

Introduction

Epidermal growth factor receptor (EGFR) is a cell-surface receptor with intrinsic intracellular protein-tyrosine kinase (TK) activity. Ligand binding induces EGFR dimerization and phosphorylation, leading to the activation of EGFR signaling pathway. In several malignancies such as non-small cell lung cancer (NSCLC), EGFR signaling is deregulated due to mutations in EGFR, which results in uncontrolled proliferation and migration of tumor cells. EGFR mutations can lead to "oncogene-addicted" cancers, where the tumor cells depend on the mutated EGFR for cell survival and the malignant phenotype. One of the most common EGFR mutations found in human patients is exon 19 deletion, within the kinase domain of EGFR. Another clinically relevant mutation associated with acquired gefitinib and erlotinib resistance is T790M, found in exon 20. Cells expressing EGFR with both L858R and T790M mutations are resistant to induced apoptosis in the presence of gefitinib or erlotinib. Three stably expressing HA-tagged EGFR HCC827 cell lines have been established—EGFR (E19del) mutant, E19del+T790M EGFR double mutant—and HCC827 cell line expressing empty vector. These cell lines can be used to study the molecular mechanism underlying the susceptibility of tumors to the drugs (i.e. gefitinib and erlotinib) as well as screening and validating new TKIs.

Materials provided

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

Handling cells upon arrival

It is strongly recommended to propagate the cells by 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 may results in a decreased responsiveness over time in normal cell culture conditions**.

Required Cell Culture Media

- **Complete Growth Media**
  In 450mL of RPMI medium, add 50mL FBS (10% final), and 5mL Penicillin/Streptomycin (1% final)

- **2x Freezing Media**
  Add 10% DMSO (final) to Complete Growth Media and sterile filter. Make fresh each time.

Materials required but not provided

(Can be substituted with a comparable third-party product)

- RPMI-1640 Medium (RPMI)) -- *Hyclone P/N SH30027.01*
- Fetal Bovine Serum (FBS) -- *Fisherbrand P/N 03-600-511*
- Penicillin/Streptomycin -- *Hyclone P/N SV30010*
- DMSO -- *Sigma P/N D8418*
- G418 antibiotic

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 T-25cm^2 flask (or 100mm^2 dish) containing 10-12ml of **Complete Growth Media.**
3. Gently rock the flask to ensure the cells are mixed well in the media. DO NOT PIPET.
4. Place the flask with cells in a humidified incubator at 37°C with 5% CO_2.
5. After cells adhere (wait at least 6 hours to overnight), **replace media** with fresh **Complete Growth Media**.

Subculture Procedure

1. Subculture/passage cells when the density reaches 90-100% confluency.
2. Carefully remove the culture media from cells by aspiration.
3. Rinse cells with PBS, being careful to not dislodge attached cells. Then remove PBS by aspiration.
4. Add 1-2 mL trypsin/Tris-EDTA solution. DO NOT add directly to cells.
5. Incubate with trypsin for 2-5 minutes (or until detached). Confirm detachment by observation under the microscope.
6. Add 5-10ml of pre-warmed Complete Growth Media and gently pipet up and down to break the clumps. Optional: for long-term maintenance, you may want to add 200-300 ug/ml G418 to the plate.
7. Passage cells in 1:3 to 1:5 ratio when they reach 90% confluency.

NOTE: Stable cell lines may exhibit a slower proliferation rate compared to parental cells. Do not seed cells at suboptimal density as this may hinder cell growth and division.

Preparing frozen stocks

This procedure is designed for 100mm^{2}dish or T75cm^2 flask. Scale volumes accordingly to other vessels.

1. When cells reach 90-100% confluency, freeze down cells.
2. Detach cells according to "Subculture Procedure."
3. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 250 x g (or 2,000 RPM) for 5 minutes to collect the cells into a pellet.
4. Carefully aspirate the media and resuspend cells in 0.5mL complete growth media.
5. Add 0.5mL of 2X freezing media and gently resuspend by pipetting up and down.
6. Transfer 1mL of cells into a cryogenic vial.
7. Place the cryogenic vial in a freezing container (*Nalgene # 5100-0001*) and store it at -80°C freezer overnight.
8. Transfer cells to liquid nitrogen for long-term storage.

**Signosis products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to this user manual or product information sheet and shipped directly by Signosis.