Signosis
Innovative Plate Assay Solutions

Epidermal growth factor receptor (EGFR) Stable Cell Line

(For Research Use Only)

EL-016 - EGFR L858R Stably Expressing CHO Cell Line
EL-017 - EGFR T790M Stably Expressing CHO Cell Line

Introduction

Epidermal growth factor receptor (EGFR) as targeted therapy has attracted much attention. 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.

Two common EGFR mutations found in human patients are deletions in exon 19 (Del19) and L858R substitution in exon 21. Patients with these mutations are sensitive to first (gefitinib or erlotinib) and second (afatinib) generation EGFR tyrosine kinase inhibitors (TKIs), whereas patients with wild type EGFR are not. However, essentially all patients become resistant to first and/or second-generation EGFR TKIs. acquired The T790M secondary mutation in exon 20 accounts for more than 50% of the acquired resistance. Cells expressing EGFR with either L858R+ T790M or Del19+T790M double mutations are resistant to induced apoptosis in the presence of first- or second-generation EGFR TKIs. Recently, third-generation EGFR TKIs such as rociletinib and osimertinibhave been developed to target T790M. Insertions in exon20 (Ins20) account for 5-10% of all EGFR mutations and indicate primary resistance to all generations of EGFR TKIs. However, a rare A763_Y764insFQEA is shown to be sensitive to the first and second generation of EGFR TKIs.

Eight cell lines stably expressing HA-tagged EGFR have been established in the IL3-dependent Ba / F3 cell lines —WT EGFR, L858R EGFR mutant, L858R/T790M EGFR double mutant, Del19 (del747-752) EGFR mutant, Del19/T790M EGFR mutant, three ins20 (D770_N771insSVD, A763_Y764insFQEA, A767_dupASV) EGFR mutants —and Ba/F3 cell line expressing empty vector. Also, two cell lines stably expressing EGFR L858R and T790M have been established in CHO cell lines.

Due to their proliferation upon EGFR activation, sensitivity to TKIs, or resistance to induced apoptosis, these cell lines can be used to study the molecular mechanism underlying susceptibility of tumors to the drugs as well as screening and validating new TKIs.

Materials provided

One vial of 2-3 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: Please thaw and culture the cells upon arrival**. Also, an adequate number of frozen stocks must be made from early passages as cells will undergo genotypic changes. Genetic instability in transfected cells will results in a decreased responsiveness over time in normal cell culture conditions.

Required Cell Culture Media

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

- **1x 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)

- DMEM -- *Hyclone SH30243.01*
- Fetal Bovine Serum (FBS) -- *Fisherbrand P/N 03-600-511*
- Trypsin -- *Hyclone P/N SH30236.02*
- Penicillin/Streptomycin -- *Hyclone P/N SV30010*
- DMSO -- *Sigma P/N D8418*

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 8-12ml 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 this incubation time (wait at least 6 hours to overnight), **replace media** with fresh **Complete Growth Media**.

Subculture Procedure

1. Subculture/passage cells when density reaches 0.8-1x10^6/ml
2. Passage cells every 3 days by inoculating 5x10^5 or in 1:3 to 1:5 ratio with warm Complete Growth Media.

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 1x10^6/ml, freeze down cells.
2. Centrifuge culture at 1000 RPM for 5 minutes to collect the cells into a pellet.
3. Carefully aspirate the media. Resuspend cells at a density of 3x10^{6}cells/ml in freshly prepared 1X freezing media and gently resuspend by pipetting up and down.
4. Aliquot 1ml cells into a cryogenic vial.
5. Place the cryogenic vial in a freezing container (*Nalgene # 5100-0001*) and store it at -80°C freezer overnight.
6. Transfer cells to liquid nitrogen for long-term storage.

Available EGFR Stable cell lines

- Control Cell line for overexpression EGFR Ba/F3 Stable Cell Line – catalog number EL-001
- EGFR (wild type) Overexpression of Ba/F3 Stable Cell Line – catalog number EL-002
- EGFR (L858R) Overexpression of Ba/F3 Stable Cell Line – catalog number EL-003
- EGFR (L858+T790M) Overexpression of Ba/F3 Stable Cell Line – catalog number EL-004
- EGFR (D770_N771insSVD) stably Expressing Ba/F3 stable cell line EL-008
- EGFR (A763_Y764insFQEA) stably Expressing Ba/F3 cells expressing EL-009
- EGFR (A767_dupASV) stably expressing Ba/F3 cells EL-010
- EGFR (DEL19) stably expressing Ba/F3 cells) EL-011
- EGFR (Del19-T790M) stably expressing Ba/F3 stable cell line EL-014

See here for a complete list of EGFR cell lines:
http://www.signosisinc.com/subcategory/egfr-stably-expressing-cell-lines

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