Signosis
Innovative Plate Assay Solutions

Human Epidermal Growth Factor Receptor 2 (HER2) Stable Cell Line

(For Research Use Only)

HER2 (WT) stably Expressing BaF3 cells - catalog number EL-031
HER2 (S310F) stably Expressing BaF3 cells – catalog number EL-032
HER2 (A775_G776insYVMA) stably Expressing BaF3 cells – catalog number EL-033
HER2 (P780_Y781insGSP) stably Expressing BaF3 cells – catalog number EL-034
HER2 (P95) stably Expressing BaF3 cells – catalog number EL-035
HER2 (A775_G776insYVMA, C805S) stably Expressing BaF3 cells – catalog number EL-044

Introduction

The human epidermal growth factor receptor (HER) tyrosine kinase family consists of ERBB1/EGFR/HER1, ERBB2/HER2, ERBB3/HER3, and ERBB4/HER4. These receptors play an important role in cellular processes including growth, proliferation, differentiation, and survival. ERBB receptors contain an extracellular domain, a transmembrane domain and a kinase domain, and a carboxy-terminal tail domain.

Overexpression and amplification of the HER2 gene (ERBB2) have been observed in about 20% of human breast and gastric cancers and therefore it has made HER2 a major target for therapy. In recent years three HER2 antibody drugs trastuzumab, adotrastuzumab emtansine, and pertuzumab, and two small molecule HER2 kinase inhibitors lapatinib and neratinib have been approved by the FDA for use in the clinic.

In addition to overexpression, some of the mutations in HER2 also play a major role in HER2-driven tumorigenesis. For instance, the kinase domain mutant G776insYVMA enhances downstream HER2 signaling and confers cellular sensitivity to trastuzumab, which is capable of downregulating both the wild-type and G776insYVMA mutant proteins from the plasma membrane.

Signosis has generated several HER2 mutant cell lines that 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 vial 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 RPMI medium, add 50mL FBS (10% final), and 5mL Penicillin/Streptomycin (1% final).

  IL3 instruction:
  - **For EL-0031** add Murine IL3 to medium at a final concentration of 10ng/ml.
  - For the rest of the above cell lines add Murine IL3 to medium at a final concentration of 1ng/ml.

- **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)

- 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*
- Murine IL-3—*Peprotech P/N 213-13*
- 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^{o}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^{o}C freezer overnight.
6. Transfer cells to liquid nitrogen for long-term storage.

Other related 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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