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Product Information

A549 Cells EGFR SH2 Biosensor

Catalog Number **CLL1097**
Storage Temperature –196 °C (liquid nitrogen)

Synonym: Human Lung Carcinoma Cell Line with GFP-tagged SH2 Biosensor

Product Description
Receptor tyrosine kinases (RTKs) such as EGFR are cell-surface growth-factor receptors that dimerize and autophosphorylate upon ligand binding. They transduce signals across the cell membrane and thus regulate diverse cell functions. In contrast to biochemical assays or immunostaining, using a natural domain-based genetically encoded biosensor allows detection of RTK activation in live cells.

The EGFR Biosensor consists of the SH2 domain of the adaptor protein Grb2 fused to GFP (see Figure 1). The biosensor was transduced into the A549 cell line and a stable cell line was cloned from a single cell. Within minutes after stimulation of the cell line with EGF, the biosensor shows robust redistribution towards the cell membrane and subsequent internalization through endocytosis (see Figure 2).

The internalization kinetics of the sensor-receptor complex can be quantified by image analysis software to count granules. The reporter response is highly selective as it can be induced only by specific EGFR ligands and is abolished by a selective inhibitor of EGFR, Tyrphostin AG 1478.

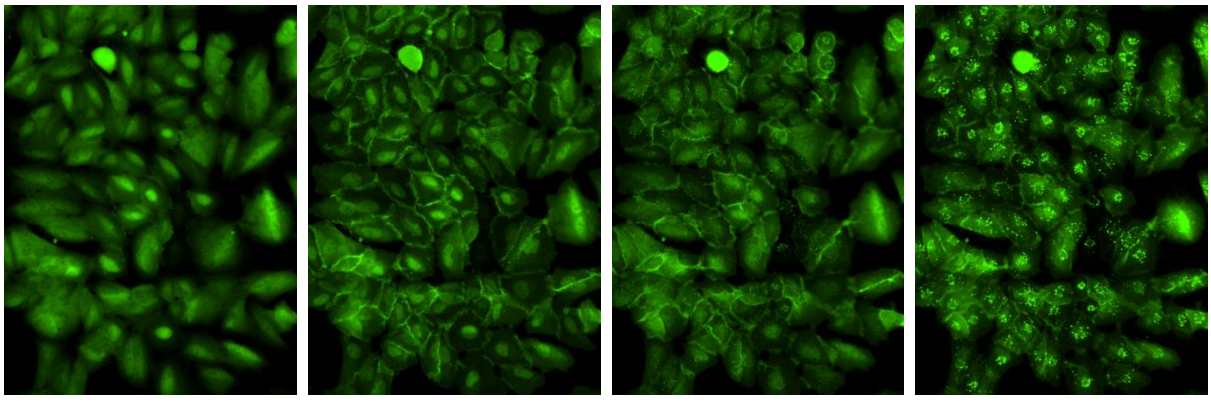
For further information go to the website: www.wherebiobegins.com/biocells

GFP and TagGFP are synonymous for the fluorescent reporter gene in this document. The GFP used in this cell line originated from Evrogen, referred to as TagGFP: http://evrogen.com/products/TagFPs.html

Figure 1.
Structure of EGFR Biosensor

The EGFR Biosensor consists of two SH2 domains from adapter protein Grb2 that specifically bind to activated EGFR and a green fluorescent protein tag (TagGFP).

Figure 2.
Response of EGFR Biosensor to EGF



EGF (0 min) **EGF (4 min)** **EGF (24 min)** **EGF (55 min)**

A lentiviral construct of tagGFP:2X$(SH2)_{Grb2}$ was used to transfect A549 cells. Single cell clones were selected and assayed for activity with EGF. This clone was homogenous for expression and EGF activity – 20x/0.75 objective, [EGF] = 100 ng/mL.

Cell Line Description
1 vial of modified A549 cells contains ~2×10^6 cells.

Organism*: Homo sapiens* (human)

Organ: lung

Age: 58 years

Gender: Male

Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile
Short Tandem Repeat (STR) analysis:
Amelogenin: X,Y
CSF1PO: 10,12
D13S317: 11
D16S539: 11,12
D5S818: 11
D7S820: 8,11
THO1: 8,9.3
TPOX: 8,11
vWA: 14

Parental Cell Line: ATCC® Catalog No. CCL-185™
Note: Please see CCL-185 product datasheet from ATCC for additional information about the origin of these cell lines. Cytogenetic information is based on initial seed stock at Sigma Life Science. Cytogenetic instability has been reported in the literature for some cell lines.

Medium: Fetal bovine serum, Catalog No. F2442, at a final concentration of 10% (v/v) in RPMI-1640 Medium, Catalog No. R0883, containing 2 mM L-glutamine, Catalog No. G7513, 1 μg/mL puromycin, Catalog No. P9620. This medium is formulated for use with a 5% CO_2 in air atmosphere.

The cryoprotectant medium used is 1× Cell Freezing Medium-DMSO, Catalog No. C6164.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Biosafety Level: 1
This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. All animal products used in the preparation and maintenance of this cell line have been screened negative by 9CFR for adventitious viral agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens. Appropriate safety procedures are recommended to be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures have been published.[1-4]

Preparation Instructions
Complete Medium: To make the complete growth medium, add fetal bovine serum, Catalog No. F2442, to a final concentration of 10% (v/v) in the base medium, RPMI-1640 Medium, Catalog No. R0883. Also add L-glutamine, Catalog No. G7513, to a final concentration of 2 mM, and puromycin, Catalog No. P9620, to a final concentration of 1 μg/mL. This medium is formulated for use with a 5% CO_2 in air atmosphere.

Storage/Stability
Upon receiving a shipment of frozen cells it is important the end user gives the shipment attention without delay. To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70 °C. Storage at –70 °C will result in loss of viability. It is highly recommended that upon initial thaw and culturing of the cells, they are expanded to allow for preparation and freezing of several additional vials of EGFR Biosensor cells to serve as backup stocks for future laboratory experiments. (See recommended Cell Freezing Medium above.)

Precaution: It is recommended that protective gloves and clothing always be used, and a full face mask always be worn when handling frozen vials. It is **important to note that some vials leak when submersed in liquid nitrogen** and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to the gas phase may result in the rapid expansion of the vessel, potentially blowing off its cap with dangerous force creating flying debris.

At the time a cell line is ordered, end users should also consider the culture conditions for the new cell line and make sure the appropriate medium will be available when the cells arrive.

Procedures

Thawing of Frozen Cells

1. Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (~2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL of Complete Medium and spin at 200 × g for 5 minutes.
4. Resuspend cell pellet with the Complete Medium and dispense into a 25 cm^2 or a 75 cm^2 culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested, prior to the addition of the vial contents, the culture vessel containing the Complete Medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0–7.6) and temperature (37 °C).
5. Incubate the culture at 37 °C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended for the Complete Medium.

Subculturing Procedure

Volumes used in this procedure are for a 75 cm^2 flask; proportionally reduce or increase volume of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Hanks (Catalog No. H6648)
3. Add 3.0 mL of Trypsin-EDTA solution to flask and incubate at 37 °C for 5 minutes to detach the cells.
4. Add 3.0 mL of Complete Medium and aspirate cells by gentle pipetting. Transfer to a centrifuge tube.
5. Rinse the flask with 5 mL of Complete Medium and aspirate by gentle pipetting. Transfer to the centrifuge tube.
6. Spin at 200 × g for 5 minutes.
7. Resuspend the cells in 10 mL of Complete Medium.
8. Add appropriate aliquots of the cell suspension into new culture vessels.
   Subcultivation Ratio: 1:3 to 1:20
9. Incubate cultures at 37 °C.

Note: It is recommended to avoid allowing the cells to become more than 60% confluent in their culture flasks to maximize the stability of the EGFR Biosensor. More information on enzymatic dissociation and subculturing of cell lines is available in the literature.[5]

Results

Specificity of the A549 EGFR Biosensor activity was tested in the cell line by visualization of the response to various ligands using fluorescence microscopy. (See Table 1 for key reagents used in these studies.) Results are shown in Figure 3 and Table 2. The cells were imaged in Hanks balanced salt solution (Catalog No. H8264) supplemented with 2% fetal bovine serum (Catalog No. F2442).

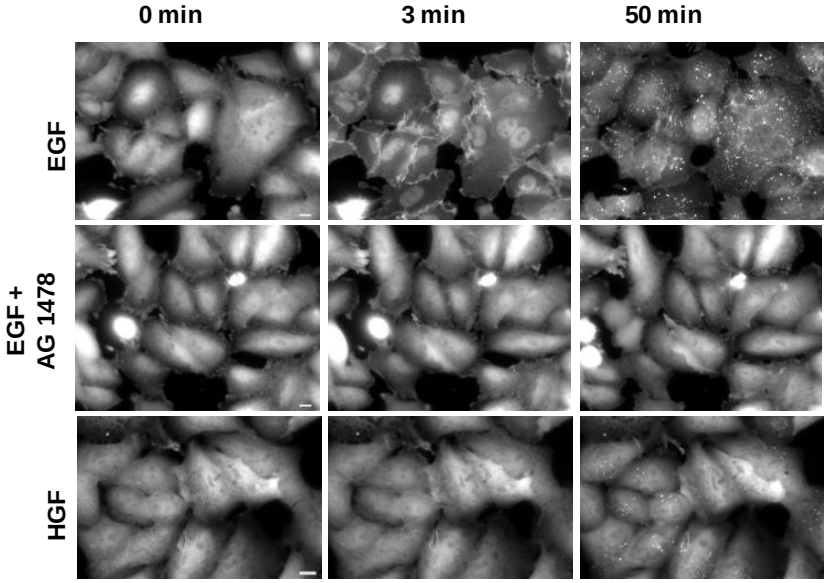
Table 1.
Key reagents used in EGFR Biosensor activity assessment.

Reagent	Catalog Number
Hanks' Balanced Salt Solution	H8264
Fetal Bovine Serum	F2442
Epidermal Growth Factor	E9644
Transforming Growth Factor-α	T7924
Hepatocyte Growth Factor	H9661
Tyrphostin AG1478	T4182

The addition of 100 ng/mL of either EGF (Catalog No. E9644) or TGF-α (Catalog No. T7924) to the cells showed significant translocation of the fluorescent biosensor to the plasma membrane followed by internalization (punctate granules).

Preincubation with 1 μM Tyrphostin AG 1478 (Catalog No. T4182), a known EGFR antagonist, for 20 minutes prior to the addition of EGF completely inhibited cellular activation of EGFR. Notably, HGF (Catalog No. H9661) exhibited a small amount of internalization activity, but all other ligands did not (Table 2).

Figure 3.
Specificity of the A549 EGFR Biosensor activity - response to various ligands



Tyrphostin AG 1478, a selective EGFR inhibitor, blocked translocation of the biosensor to the plasma membrane and subsequent internalization. HGF, a ligand specific for HGFR showed much less activity than EGF.

Table 2.
Specificity of the A549 EGFR Biosensor activity - response to various ligands

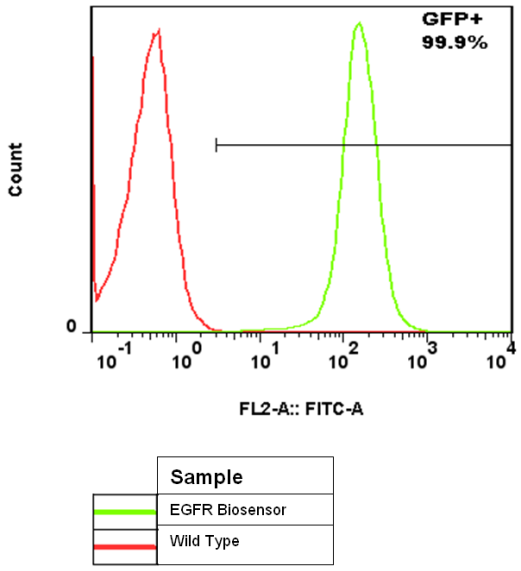
Ligand	Activity
EGF	++++
TGF-α	++++
HGF	+
Heregulin-β1	–
PDGF-AB	–
Insulin	–
IGF-1	–
NGF-β	–
FGF-acidic	–
Angiopoietin	–
MSP	–
Gas6	–
VEGF	–
FLT-3	–

A panel of ligands to receptor tyrosine kinases was tested for activity with the biosensor cells. Strong activities were only seen with the EGFR-specific ligands, EGF and TGF-α. Heregulin-β1, IGF-1, and Gas6 were tested at 1 μg/mL. Insulin was tested at 2 μg/mL. All other ligands were tested at 100 ng/mL.

Stability of EGRF Biosensor Expression

The population of cells exhibiting high expression of the EGFR Biosensor construct were evaluated by flow cytometry analysis (see Figure 4) and were shown to be >99% after 2 weeks in culture and >90% after 4 weeks in culture. Due to the random nature of the lentiviral integration, it is expected the percentage of high expressing cells will decrease during extended periods in culture. Therefore, it is highly recommended to prepare additional stocks of frozen cells by expansion of the original vial received upon initial thaw and culturing of the EGFR Biosensor cells.

Figure 4.
Flow cytometry analysis of the EGFR Biosensor cell line showed a homogeneous population



References

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4. Centers for Disease Control (1999), Biosafety in Microbiological and Biomedical Laboratories Human Health Service Publication No. (CDC) 93-8395. U.S. Dept. of Health and Human Services; 4th Edition U.S. Government Printing Office Washington D.C. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm
5. Freshney, R.I., Chapter 10 in Culture of Animal Cells, a manual of Basic Technique by, 3rd edition, published by Alan R. Liss, (NY, NY: 1994).

Additional product and technical information can be obtained from the catalog references and the Sigma Life Science Website (www.wherebiobegins.com/biocells).

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