Signosis
Innovative Plate Assay Solutions

Luciferase stably expressing HT29 stable cell line

Catalog number SL-0111 (For Research Use Only)

Introduction

Luciferase stably expressing HT29 cell line is derived from human colorectal adenocarcinoma. The cell line was established by transfection of the luciferase expression vector along with hygromycin expression vector followed by hygromycin selection. The hygromycin-resistant clones were subsequently screened for expression. The clone with the highest expression was selected and expanded to produce this stable cell line. This cell line can potentially be used to develop a luciferase activity-based assay, as a positive control for luciferase activity, or to test if experimental conditions affect luciferase expression.

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Provided Materials

One vial of 2 x 10^6 cells, at passage 4, 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 that you propagate the cells by following instructions as soon as possible upon arrival **.

IMPORTANT: It is imperative that an adequate number of frozen stocks 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-1640, 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.

Materials required but not provided

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

Materials	Product number
Dulbecco's Modified Eagles Medium (DMEM)	Hyclone SH30243.01
Fetal Bovine Serum (FBS)	Fisherbrand P/N 03-600-511
Penicillin/Streptomycin	Hyclone P/N SV30010
Trypsin	Hyclone P/N SH30236.02
Phosphate-buffered saline (PBS)	Cellgro P/N 21-040-CV
DMSO	Sigma P/N D8418
96-well white plate	Greiner Bio-One P/N 655098
Luciferase substrate	Signosis P/N LUC015
Cell lysis buffer	Signosis P/N LS-001
Hygromycin B (Optional)	Toku-E P/N H010

Initial Culture Procedure

1. Quickly thaw cells in a 37 ºC 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 ºC 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 accordingly 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 (e.g. *Nalgene # 5100-0001*) and store it at -80ºC freezer overnight.
6. Transfer cells to liquid nitrogen for long-term storage.

Assay procedure

1. The day before performing the assay, trypsinize the cells and seed each well of a **white clear-bottom** 96 well plate with 1-3 x 10^4 cells in 100µl.
2. Incubate the plate in a humidified incubator at 37ºC with 5% CO_2 overnight.
3. Remove the media by aspiration and add 100µl of PBS to each well.
4. Remove PBS by aspiration and add 20µl of 1x lysis buffer to each well (To prepare 1x lysis buffer, add one volume of 5x lysis buffer to four-volume of distilled water).
5. Incubate cells in lysis buffer for 20 minutes at room temperature with gentle agitation.
6. Add 100µl of luciferase substrate to each well and gently pipette up and down.
7. Immediately read the plate in a luminometer.

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