Signosis
Innovative Plate Assay Solutions

P53 Stably Expressing Cos-7 Cell Line

Catalog number SL-0100 (For Research Use Only)

Introduction

p53 plays a key role in cellular homeostasis and is at the heart of a complex network of protective mechanisms safeguarding cellular integrity. Because of its central function in processes such as cell cycle regulation, apoptosis, DNA repair, cellular senescence, and apoptosis, the p53 pathway is crucial for effective tumor suppression. Signosis has established a stable cell line with constitutive overexpression of wild-type p53 with myc tag in Cos-7 cell. Therefore, the cell line can be used as an expression system for monitoring the function of p53 in different applications, such as activation and protein-protein interactions.

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: 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, 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

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 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 °C 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.

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