Signosis
Innovative Plate Assay Solutions

NFkB (p65) Stably Expressing Cos-7 Cell Line

Catalog number SL-0101 (For Research Use Only)

Introduction

NF-kB is composed of homo- and heterodimers of five members of the Rel family including NF-kB1(p50), NF-kB2 (p52), RelA (p65), RelB, and c-Rel (Rel). The major dimer, RelA(p65)/p50 is sequestered in the cytosol of unstimulated cells via IKBs. Signal induction leads to IkB dissociation and degradation and allows activation of the NF-kB complex. The activated NF-kB complex translocates into the nucleus, and binds DNA at kB-binding motifs, and initiates the target gene transcription. The NFkB plays an important role in many biological processes, such as differentiation, proliferation, and apoptosis. The dysfunction of NFkB causes many diseases, such as inflammation and cancer. Signosis developed RelA(p65) overexpression stable Cos-7 cell line, allowing study NFkB pathway effectively.

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.

** Signosis products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to this user manual or product information sheet and shipped directly by Signosis.