

Product data sheet

A375 SMAD/TGFbeta Reporter (Luc) stable cell line

Catalog Number: CL-1464

Storage: Liquid nitrogen

Components: 1 vial contains $\sim 2 \times 10^6$ cells in Cell freezing medium

Product description

A375 SMAD/TGFbeta Reporter (Luc) cells are derived from the human melanoma A375 cell line by stably integration of a SMAD/TGFbeta firefly luciferase reporter construct. A375 cell line has been widely used in cancer research and drug development. A375 SMAD/TGFbeta Reporter (Luc) cells stably express firefly luciferase under the control of the SMAD/TGFbeta response elements, can be used for monitoring the activity of TGF β /SMAD signaling pathway.

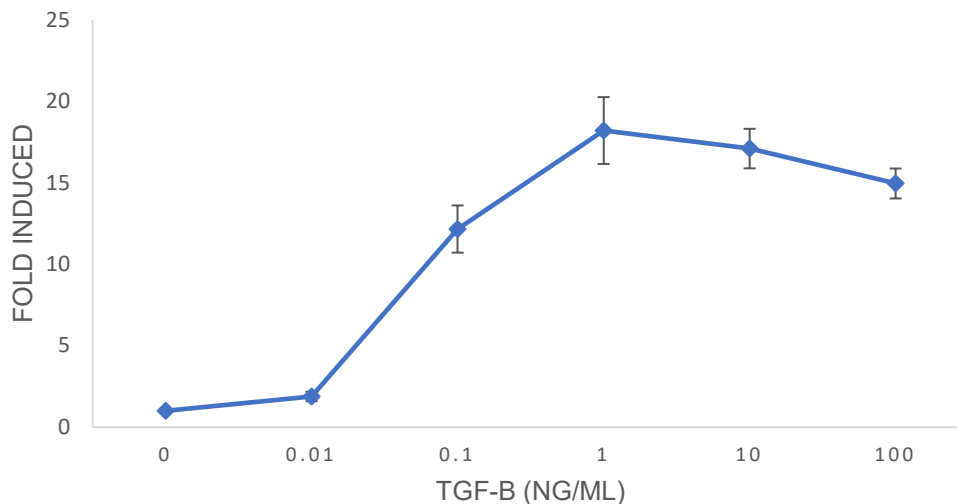


Figure 1. Dose-response of A375 SMAD/TGFbeta Reporter (Luc) cells to recombinant human TGF- β . Cells were stimulated with increasing concentrations of recombinant human TGF- β 1 (hTGF- β 1). After incubation for 24 hours, the TGF- β /Smad response was determined using Bright-GloTM luciferase Assay System (Promega, Cat E2610).

Cell line description

Organism: Homo sapiens (human)

Tissue: Skin

Morphology: epithelial

Culture Properties: adherent
Disease: Malignant Melanoma
Biosafety Level: 2

Medium

1. Complete culture medium: DMEM with 10% fetal bovine serum (FBS)
1 µg/mL of puromycin may be added to the culture medium. **Puromycin should not be added until a culture has been well established from the thawed cells.**
2. Freeze medium: FBS with 6% DMSO

Culture procedure

Thawing of frozen cells

1. Thaw the frozen cryovial by gentle agitation in a 37 °C water bath in 1-2 minutes.
2. Remove the cryovial from the water bath as soon as the contents are thawed, and decontaminate by wiping with 70% ethanol.
3. Transfer the thawed cell suspension to a centrifuge tube containing 10 ml of Complete culture medium, centrifuge at 500 g for 5 minutes.
4. Remove the medium by aspiration, resuspend the cells with 10 ml of the Complete culture medium by gently pipetting up and down.
5. Transfer the cells to a 10 cm cell culture dish.
6. Place the cells in a 37°C incubator with 5% CO₂.

Sub-culturing

Volumes are given for a 10 cm cell culture dish. Increase or decrease the amount of dissociation medium needed proportionally.

1. Remove the medium by aspiration.
2. Briefly rinse the cell layer with 1xDPBS to remove all traces of serum that contains trypsin inhibitor.
3. Add 1 ml of Trypsin-EDTA (0.25%) solution to the dish and observe cells under an inverted microscope until cell layer is dispersed.
4. Add 4 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C with 5% CO₂.