INTRODUCTION

The eLUCidate™ SBE Reporter cell line is a stably transfected HEK 293 cell line which expresses Renilla luciferase reporter gene under the transcriptional control of the SMAD binding element (SBE). SMADs are intracellular signaling mediators that transduce extracellular signals from transforming growth factor beta (TGF-beta) ligands to the nucleus where they activate downstream gene transcription. The TGF-beta signaling pathway is involved in many cellular processes in both the adult organism and the developing embryo including cell growth, cell differentiation, apoptosis, cellular homeostasis and other cellular functions. The SBE induction by TGF-beta is shown in Figure 1 below.

MATERIALS AND METHODS

1. General Culture Conditions

   Cells should be grown at 37°C with 5% CO₂ using Growth Medium (DMEM medium supplemented with 10% FBS, 1% Pen/Strep, plus 3 µg/ml Puromycin). Users should also prepare Growth Medium without Puromycin for early stage cell thawing and growth.

2. Thawing and Growing Cells

   2.1 Quickly thaw frozen cell upon receipt (or from liquid nitrogen storage) in a 37°C water bath.
   2.2 Transfer to a tube containing 10 ml of Growth Medium without Puromycin.
   2.3 Spin cells down at 1,000 rpm for 10 minutes.
   2.4 Gently resuspend cells in pre-warmed Growth Medium without Puromycin.
   2.5 Transfer resuspended cells to a T25 flask and culture in a 37°C CO₂ incubator.
   2.6 Leave the T25 flask in the incubator for 2-4 days without disturbing or changing the medium, and until cells completely recover viability and become adherent.
   2.7 Once cells are over 90% confluent, harvest by using Detachin™ Cell Detachment Solution (Cat # T100100), and centrifuge to collect cells.
   2.8 Replate cells and passage as needed; as first passage and after, switch to Growth Medium containing Puromycin.

   NOTE: cells should be split before reaching complete confluence.

3. Cell Passaging

   3.1 Detach cells using Detachin™ Cell Detachment Solution (Cat #: T100100).
   3.2 Add 10 ml Growth Medium to a sterile 50 ml centrifuge tube.
   3.3 Pellet cells by centrifuging at 1,000 rpm for 10 min.
   3.4 Resuspend cells in Growth Medium to achieve 1:10 or 1:20 dilution ratio, and plate as needed.
4. Functional Validation: Response of eLUCidate HEK 293, SBE to transforming growth factor beta (TGF-β)

4.1 Harvest and seed cells into a white solid-bottom 96-well microplate in 100 μl of Growth Medium at 5 x 10^4 cells/well.

4.2 Incubate cells at 37°C in a CO_2_ incubator overnight.

4.3 On next day, stimulate cells with different concentrations of TGF-β.

4.4 Incubate at 37°C in a CO_2 incubator for 6-16 hours.

4.5 Add 50 μl of a luciferase assay reagent per well.

4.6 Incubate at room temperature for 1-5 minutes and measure luminescence using a microplate luminometer.

Figure 1: Induction of SBE activity by TGF-β in eLUCidate™ HEK 293 SBE cells.

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