INTRODUCTION

The eLUCidate NFAT reporter cell line is a stably transfected HEK 293 cell line which expresses Renilla luciferase reporter gene under the transcriptional control of the Nuclear Factor of Activated T-cells (NFAT) response element. NFAT is a transcription factor originally found in activated T lymphocytes, and is now known to regulate not only T cell activation and differentiation but also the function of other immune cells including dendritic cells, B cells and megakaryocytes. The eLUCidate NFAT reporter cell line is tested by induction with induction by calcium ionophore A23187, and provides an effective way to monitor or screen for activators/inhibitors of the NFAT signaling pathway.

MATERIALS AND METHODS

1. General Culture Conditions

Cells should be grown at 37°C with 5% CO₂ using Growth Medium (DMEM plus 10% FBS and 1% Pen/Strep). Where needed, add 3 μg/ml Puromycin.

2. Thawing and Growing Cells

2.1 Aliquot 10 ml of Growth Medium to a 15 ml sterile tube.
2.2 Remove cells from storage and quickly thaw in a 37°C water bath.
2.3 Transfer cells to the tube in 2.1.
2.4 Spin cells down at 1,000 rpm for 10 minutes.
2.5 Resuspend cells in pre-warmed Growth Medium.
2.6 Transfer cells to a T25 flask (or equivalent) and culture at 37°C in a CO₂ incubator.
2.7 At first passage, transfer cells into Growth Medium with Puromycin.
2.8 Split cells when they reach about 80-90% confluence.

3. Cell Passaging

3.1 Detach cells using Detachin™ Cell Detachment Solution (Cat #: T100100).
3.2 Add 10 ml Growth Medium with Puromycin to cells and transfer 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 with Puromycin to achieve 1:10 or 1:20 dilution ratio, and plate as needed.

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