INTRODUCTION
The eLUCidate Nrf2 reporter cell line is a stably transfected MCF7 cell line which expresses Renilla luciferase reporter gene under the transcriptional control of the antioxidant response element (ARE). ARE is known to regulate expression and induction of various detoxifying enzyme genes in response to antioxidants and xenobiotics, and is primarily regulated by the Keap1-Nrf2 pathway in which induction and nuclear translocation of Nrf2 mediated by antioxidants and xenobiotics results in the binding of Nrf2 to ARE leading to the expression of defensive genes. One of the antioxidants, curcumin, is known to upregulate Nrf2 leading to activation of the AREs. The eLUCidate Nrf2 reporter cell line is tested by induction with curcumin, and provides an effective way to monitor or screen for activators/inhibitors of the Nrf2 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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