INTRODUCTION

The eLUCidate™ MRE Reporter cell line is a stably transfected HEK 293 cell line which expresses Renilla luciferase reporter gene under the transcriptional control of the MRE-binding transcription factor-1 (MTF-1) which is a zinc finger transcription factor and plays a major role in induction of metallothionein gene expression in response to cellular stress caused by heavy metals such as zinc and cadmium. The MRE induction by ZnSO$_4$ is shown in Figure 1 below.

MATERIALS AND METHODS

1. General Culture Conditions

   Cells should be grown at 37°C with 5% CO$_2$ 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 CO$_2$ 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.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Content</th>
<th>Amount</th>
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<tbody>
<tr>
<td>EL-MRE293</td>
<td>eLUCidate™ HEK 293, MRE Reporter Cell Line</td>
<td>2-3 x 10$^6$ cells in 1 ml</td>
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<td>90% FBS, 10% DMSO</td>
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Shipping: Dry ice

Storage: Liquid nitrogen
4. Functional Validation: Response of eLUCidate™ HEK 293, MRE to Zinc Sulfate (ZnSO₄)

4.1 Harvest and seed cells into a white solid-bottom 96-well microplate in 100 µl of Growth Medium at 5 x 10⁴ cells/well.

4.2 Incubate cells at 37°C in a CO₂ incubator overnight.

4.3 On next day, stimulate cells with different concentrations of ZnSO₄.

4.4 Incubate at 37°C in a CO₂ 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.

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**Figure 1:** Induction of MRE activity by Zinc Sulfate in eLUCidate™ HEK 293 MRE cells.