INTRODUCTION
The eLUCidate HRE reporter cell line is a stably transfected HeLa cell line which expresses Renilla luciferase reporter gene under the transcriptional control of the hypoxia response element (HRE). In response to hypoxia (low oxygen), HREs of target genes are recognized and regulated by the hypoxia-inducible factors (HIFs) which belong to the family of basic helix-loop-helix transcription factors and form heterodimeric complex comprising the alpha subunit (HIF-1 alpha, HIF-2 alpha and HIF-3 alpha) and beta subunit (Arnt1, Arnt2 and Arnt3), among which HIF-1 alpha and HIF-2 alpha are predominant isofoms. Activation of HIFs can also be mediated by chemical hydroxylase inhibitors as hypoxia mimetics including the iron chelator desferrioxamine and cobalt chloride. The eLUCidate HRE reporter cell line is tested by induction with cobalt chloride.

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 1:1 or 1:20 dilution ratio, and plate as needed.

LIMITED LICENSE: The purchase price paid for the eLUCidate™ Reporter Cell Lines (hereo “eLUCidate”) grants end users a non-transferable, non-exclusive license to use the cells for internal research use only as described in this manual; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Genlantis -- separate licenses are available for resale or other commercial applications. eLUCidate is not to be used for human research, diagnostic applications, or included/used in any drug intended for human use. Care and attention should be exercised in handling eLUCidate by following appropriate research laboratory practices and kit instructions, including the proper use of protective lab wear during use. Purchasers may refuse this license by returning the enclosed materials unused. By keeping or using Attachin, you agree to be bound by the terms of this license as governed by the laws of the State of California.