

Luciferase Reporter Cell Lines

Cat. No.	Content	Amount
EL-MDA5293T	eLUCidate™ HEK 293T, MDA5 Reporter Cell Line	2-3 x 10 ⁶ cells in 1 ml 90% FBS, 10% DMSO

Shipping	Dry ice
Storage	Liquid nitrogen

INTRODUCTION

The eLUCidate MDA5 reporter cell line is a stably transfected HEK 293T cell line which expresses the melanoma differentiation-associated protein-5 (MDA5) and Renilla luciferase reporter gene under the transcriptional control of an NF-κB response element. As a dsRNA helicase enzyme, MDA5 is encoded by the IFIH1 gene. MDA5 is one of the RIG-I-like receptors (RLRs) that are a family of DExD/H box RNA helicases including MDA5, RIG-I and LPG2, which play a major role in pathogen sensing of RNA virus infection to initiate and modulate antiviral immunity. RLR expression is typically maintained at low levels in resting cells but is greatly increased during inflammation, specifically with IFN exposure and after virus infection. MDA5 detects cytoplasmic dsRNA generated during viral replication unlike Toll-like receptor 3 (TLR3) which can detect phagocytosed dsRNA in endosomes. MDA5 also responds to poly(I:C), the synthetic analog of viral dsRNA. The eLUCidate MDA5 reporter cell line is tested by inducing with Polyinosinic-polycytidylic acid (poly(I:C)).

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 indicated, add 3 µg/ml Puromycin and 5 µg/ml Blasticidin

2. Thawing and Growing Cells

- 2.1 Aliquot 10 ml of Growth Medium to a 15 ml sterile tube.
- 2.2 Quickly thaw cells 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 and Blasticidin.
- 2.8 Split cells when they reach about 80-90% confluence.

RELATED PRODUCTS	Catalog #
eLUCidate™ RAW 264.7, NF-κB Reporter Cell Line	EL-NFKBRAW
eLUCidate™ RAW 264.7, IL-8 Reporter Cell Line	EL-IL8RAW
eLUCidate™ HEK 293, MIP-2 Reporter Cell Line	EL-MIP2293
eLUCidate™ RAW 264.7, MIP-2 Reporter Cell Line	EL-MIP2RAW
eLUCidate™ HEK 293, TNF-α Reporter Cell Line	EL-TNFA293
eLUCidate™ RAW 264.7, TNF-α Reporter Cell Line	EL-TNFARAW
eLUCidate™ HEK 293, TNF-β Reporter Cell Line	EL-TNFB293
eLUCidate™ RAW 264.7, TNF-β Reporter Cell Line	EL-TNFBRAW
eLUCidate™ HEK 293, GATA3 Reporter Cell Line	EL-GATA3293
eLUCidate™ HEK 293, NFAT Reporter Cell Line	EL-NFAT293
eLUCidate™ RAW 264.7, NFAT Reporter Cell Line	EL-NFATRAW
eLUCidate™ HeLa, STAT1 Reporter Cell Line	EL-STAT1HELA
eLUCidate™ RAW 264.7, STAT1 Reporter Cell Line	EL-STAT1RAW
eLUCidate™ HEK 293, STAT3 Reporter Cell Line	EL-STAT3293
eLUCidate™ RAW 264.7, INOS Reporter Cell Line	EL-INOSRAW
eLUCidate™ NIH-3T3, IL-6 Reporter Cell Line	EL-IL63T3
eLUCidate™ MCF7, Nrf2 Reporter Cell Line	EL-NRF2MCF7
eLUCidate™ HeLa, HRE Reporter Cell Line	EL-HREHELA
eLUCidate™ HEK 293, TCF/LEF Reporter Cell Line	EL-LEF293T
eLUCidate™ HEK 293, AP-1 Reporter Cell Line	EL-AP1293
eLUCidate™ HEK 293T, RIG-I Reporter Cell Line	EL-RIGI293T
eLUCidate™ HEK 293, TLR3/IFNB Reporter Cell Line	EL-IFNB293
eLUCidate™ HEK 293, TLR3/ISRE Reporter Cell Line	EL-ISRE293
eLUCidate™ HeLa, TLR4/IL-8 Reporter Cell Line	EL-IL8HELA
eLUCidate™ HEK 293, NF-κB Reporter Cell Line	EL-NFKB293
eLUCidate™ HeLa, p53 Reporter Cell Line	EL-P53HELA
eLUCidate™ HEK 293, TLR2/NF-κB Reporter Cell Line	EL-TLR2293
eLUCidate™ HEK 293, TLR3/NF-κB Reporter Cell Line	EL-TLR3293
eLUCidate™ HEK 293, TLR7/NF-κB Reporter Cell Line	EL-TLR7293
eLUCidate™ HEK 293, TLR8/NF-κB Reporter Cell Line	EL-TLR8293
eLUCidate™ HEK 293, TLR9/NF-κB Reporter Cell Line	EL-TLR9293
eLUCidate™ HEK 293, SRE Reporter Cell Line	EL-SRE293
eLUCidate™ HEK 293, SRF-RE Reporter Cell Line	EL-SRFRE293
eLUCidate™ HEK 293, CRE Reporter Cell Line	EL-CRE293

3. Cell Passaging

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

LIMITED LICENSE: The purchase price paid for the eLUCidate™ Reporter Cell Lines (hereto "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.