

Luciferase Reporter Cell Lines

INTRODUCTION

The eLUCidate™ TLR5/NF-κB Reporter cell line is a stably transfected HEK 293 cell line which expresses full-length human Toll-like receptor 5 (TLR5) and Renilla luciferase reporter genes under the transcriptional control of the NF-κB response element. TLR5 is one of the key innate immune receptors. Functional activity of the cell line has been validated by TLR5 ligand assay, in which upon activation by Flagellin, TLR5 quickly initiates downstream signaling pathway and mediates nuclear translocation of NF-κB as shown in Figure 1 below.

Cat. No.	Content	Amount
EL-TLR5293	eLUCidate™ HEK 293, TLR5/NF-κB Reporter Cell Line	2-3 x 10 ⁶ cells in 1 ml 90% FBS, 10% DMSO
Shipping	Dry ice	
Storage	Liquid nitrogen	

MATERIALS AND METHODS

1. General Culture Conditions

Cells should be grown at 37°C with 5% CO₂ using Growth Medium (DMEM medium supplemented with 10% FBS, 1% Pen/Strep, plus 2 µg/ml Puromycin and 5 µg/ml of Blasticidin). Users should also prepare Growth Medium without Puromycin and Blasticidin 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 and Blasticidin.
- 2.3 Spin cells down at 1,000 rpm for 10 minutes.
- 2.4 Gently resuspend cells in pre-warmed Growth Medium without Puromycin and Blasticidin.
- 2.5 Transfer resuspended cells to a T25 flask and culture in a 37°C CO₂ 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 and Blasticidin.

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.

4. Functional Validation: Response of eLUCidate™ HEK 293, TLR5/NF-κB to Flagellin.

- 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 Flagellin.
- 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.

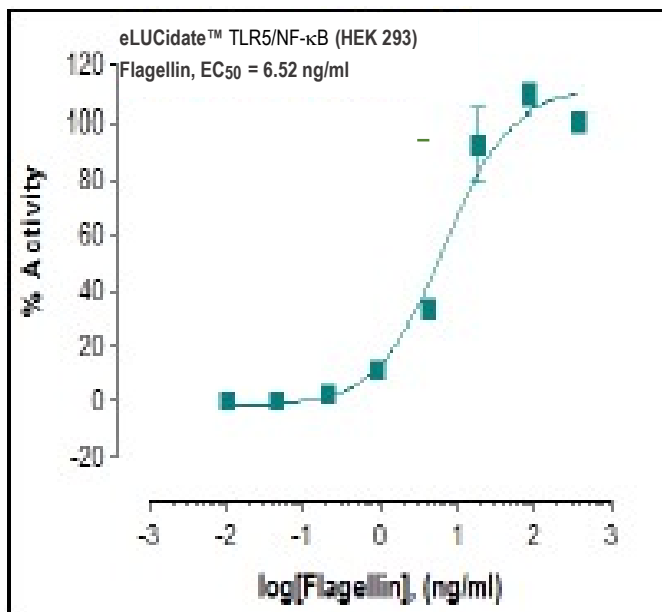


Figure 1: Induction of TLR5 activity by Flagellin in eLUCidate™ HEK 293 TLR5/NF-κB cells.

RELATED PRODUCTS	Catalog #
eLUCidate™ HEK 293, TLR9/NF-κB Reporter Cell Line	EL-TLR9293
eLUCidate™ RAW 264.7, IL-8 Reporter Cell Line	EL-IL8RAW
eLUCidate™ HEK 293, AP-1 Reporter Cell Line	EL-AP1293
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-TNFA2RAW
eLUCidate™ HEK 293, TNF-β Reporter Cell Line	EL-TNFB293
eLUCidate™ RAW 264.7, TNF-β Reporter Cell Line	EL-TNFB2RAW
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-NFAT2RAW
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 293T, RIG-I Reporter Cell Line	EL-RIGI293T
eLUCidate™ HEK 293T, MDA5 Reporter Cell Line	EL-MDA5293T
eLUCidate™ HEK 293, TLR3/IFNβ 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™ RAW 264.7, NF-κB Reporter Cell Line	EL-NFKBRAW
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

RELATED PRODUCTS	Catalog #
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, 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
eLUCidate™ Ba/F3, STAT5 Reporter Cell Line	EL-STAT5BAF3
eLUCidate™ HEK 293, SBE Reporter Cell Line	EL-SBE293
eLUCidate™ HEK 293, MRE Reporter Cell Line	EL-MRE293
eLUCidate™ HeLa, ATF6 Reporter Cell Line	EL-ATF6HELA
eLUCidate™ HEK 293, TLR5/NF-κB Reporter Cell Line	EL-TLR5293
eLUCidate™ RAW 264.7, ISRE Reporter Cell Line	EL-ISRERAW
eLUCidate™ Jurkat, NF-κB Reporter Cell Line	EL-NFKBJUR
eLUCidate™ HEK 293, ISRE Reporter Cell Line	EL-ISRE293
eLUCidate™ Jurkat, FOXP3 Reporter Cell Line	EL-FOXP3JUR
xCELLerate™ PD-1 Stable Cell Line	XCL-PD1
xCELLerate™ PD-L1 Stable Cell Line	XCL-PDL1
xCELLerate™ PD-L2 Stable Cell Line	XCL-PDL2
xCELLerate™ ICOS Stable Cell Line	XCL-ICOS
xCELLerate™ ICOSL Stable Cell Line	XCL-ICOSL
xCELLerate™ CD80 Stable Cell Line	XCL-CD80
xCELLerate™ CTLA4 Stable Cell Line	XCL-CTLA4
xCELLerate™ hB7-H4 Stable Cell Line	XCL-HB7H4
xCELLerate™ mB7-H4 Stable Cell Line	XCL-MB7H4
xCELLerate™ Langerin Stable Cell Line	XCL-LNGRN
xCELLerate™ Cas9 Stable Cell Line	XCL-CAS9
xCELLerate™ NKP30 Stable Cell Line	XCL-NKP30
xCELLerate™ NKP46 Stable Cell Line	XCL-NKP46

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 eLUCidate, you agree to be bound by the terms of this license as governed by the laws of the State of California.