



Promega

Technical Bulletin

GloResponse™
NF- κ B-RE-*luc2P* HEK293
Cell Line

INSTRUCTIONS FOR USE OF PRODUCT E8520.



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GloResponse™ NF-κB-RE-*luc2P* HEK293 Cell Line

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1. Description

Luciferase reporter assays are used widely to investigate cellular signaling pathways and as high-throughput screening tools for drug discovery (1,2). The GloResponse™ NF-κB-RE-*luc2P* HEK293 Cell Line^(a-h) is a clonal derivative of Human Embryonic Kidney 293 (HEK293)^(f) cells. These cells contain a luciferase gene (*luc2P*) under the control of a minimal TATA promoter with multiple Nuclear Factor-κB response elements (NF-κB-REs). NF-κB-REs are the DNA-binding sequences for the NF-κB transcription factor, which is responsible for regulating inflammation, immune response, cell growth and apoptosis. The GloResponse™ NF-κB-RE-*luc2P* HEK293 Cell Line is designed for rapid and convenient analysis of any cellular response that results in modulation of NF-κB activities.

The GloResponse™ NF-κB-RE-*luc2P* HEK293 Cell Line was generated by clonal selection of HEK293 cells stably transfected with the pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector (Cat.# E8491). This cell line incorporates the improvements developed for the pGL4 family of reporter vectors for enhanced performance (3). The destabilized *luc2P* luciferase reporter is used to improve responsiveness to transcription dynamics. The *luc2P* gene is codon-optimized for enhanced expression in mammalian cells, and the pGL4 plasmid backbone has been engineered to reduce background reporter expression.

2. Product Components and Storage Conditions

Product	Size	Cat.#
GloResponse™ NF-κB-RE- <i>luc2P</i> HEK293 Cell Line	2 vials	E8520

Includes: Two vials of 2×10^6 cells in Freezing Media.

- ⚠ Cells are shipped frozen. If cells are not frozen upon arrival, contact Promega customer service immediately.
- ⚠ Place frozen cells in storage at or below -140°C (mechanical deep freeze or vapor phase liquid nitrogen) until you are ready to thaw and propagate them.

WARNING: Do not use cryotubes in the liquid phase of liquid nitrogen. Improper use may trap liquefied nitrogen inside the vial and lead to pressure buildup, resulting in possible explosion or biohazard release. Use appropriate safety procedures when handling and disposing of the cryotubes.

Product Warranty

Promega warrants that cells will be viable upon shipment from Promega for a period of thirty days, provided they have been properly stored and handled during this period.

Handling Cells Upon Arrival

We strongly recommend that you propagate the cells using the provided procedure as soon as possible after receipt (Section 4). This will ensure the best cell viability and assay performance. The second vial is supplied as an immediate backup and is not intended for long-term storage.

Cell Line Stability

Cells may undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

3. Before You Begin

3.A. Materials to Be Supplied by the User

Recommended Reagents

- DMEM, high glucose (Invitrogen Cat.# 11965-118)
- characterized fetal bovine serum (FBS; Hyclone Cat.# SH30071)
- DMSO (Sigma-Aldrich Cat.# D2438)
- hygromycin B (Invitrogen Cat.# 10687-010)
- phosphate-buffered saline (PBS; Invitrogen Cat.# 20012-050)
- TrypLE™ Express trypsin (Invitrogen Cat.# 12605-010) or 0.05% Trypsin-EDTA 1X (Invitrogen Cat.# 25300-054)
- ONE-Glo™ Luciferase Assay System (Promega Cat.# E6110) or Dual-Glo® Luciferase Assay System (Promega Cat.# E2920)
- TNF α (Tumor Necrosis Factor- α ; Promega Cat.# G5241 or Sigma-Aldrich Cat.# T0157)

Supplies and Equipment

- tissue culture-treated, solid white, 96-well assay plate (Costar® Cat.# 3917)
- 15ml conical tubes
- cryovials
- tissue culture flasks
- class II biological safety cabinet
- hemacytometer
- humidified 37°C, 5% CO₂ incubator
- inverted microscope
- luminometer

3.B. Composition of Required Media and Buffers

Growth Medium

90% DMEM
10% FBS

Growth Medium + hygromycin B

90% DMEM
10% FBS
50 μ g/ml hygromycin B

Freezing Medium

80% DMEM
15% FBS
5% DMSO

Assay Medium

90% DMEM
10% FBS

TNF α Stock (500X)

10 μ g/ml in 1X PBS with
1mg/ml BSA

5X TNF α Solution

100ng/ml in Assay Medium

4. Maintenance of GloResponse™ NF-κB-RE-*luc2P* HEK293 Cell Line

4.A. Thawing and Initial Culture Procedure

1. Rapidly thaw the cells by placing them at 37°C in a water bath with gentle agitation for 1–2 minutes.
Note: Freezing Medium may be yellow immediately after thawing. This does not affect cell viability if these instructions are followed.
2. Decontaminate the vial by wiping it with 70% ethanol before opening in a class II biological safety cabinet.
3. Slowly transfer the vial contents into 10ml of Growth Medium (Section 3.B) in a sterile 15ml conical tube.
4. Centrifuge the cells at 500 × *g* for 5 minutes at 18°C.
5. Aspirate the supernatant and resuspend the cell pellet in 12ml of 37°C prewarmed Growth Medium.
6. Transfer resuspended cells to a T75 flask, and culture cells as described in Section 4.B.

4.B. Propagation of Cells

Cells should be maintained between 10% and 90% confluency in a 37°C, 5% CO₂ tissue culture incubator. This typically will require passaging the culture twice a week. The approximate cell number for 100% confluency for this cell line in a T75 flask is 1×10^7 cells. Media formulations are provided in Section 3.B. Volumes listed are for propagation in a T75 flask.

1. When cells have reached the appropriate density, aspirate the medium from the flask.
2. Wash once with 2ml 1X PBS.
3. Add 2ml of 37°C TrypLE™ Express trypsin. Evenly coat the flask surface containing the cells. Trypsinize for 2 minutes.
4. Using a microscope, verify that the cells have detached and clumps have completely dispersed.
5. Stop trypsinization by adding 10ml of Growth Medium.
6. Transfer cell suspension to a conical tube. Determine cell number using a hemacytometer.
7. Pellet cells at 500 × *g* for 5 minutes at 18°C.
8. Aspirate the supernatant and resuspend cells in Growth Medium + hygromycin B.
9. Seed new flasks at appropriate cell density, depending on the size of flask. For example, use 1×10^6 cells for a T75 flask.
10. Place flasks in 5% CO₂, 37°C incubator.

4.C. Freezing Cells

1. Grow cells to a density of 50% confluency. Replace Growth Medium + hygromycin B with Growth Medium (no hygromycin B) the day before harvest.
2. Harvest cells as described in Section 4.B. After the cells have detached, briefly centrifuge cells and resuspend them in Freezing Medium (Section 3.B).
3. Dispense 1.0ml per cryogenic vial.
4. Place vials in an insulated container (i.e., Styrofoam® or Nalgene® Mr. Frosty, Cat.# 5100-0001) for slow cooling, and store overnight at -80°C.
5. Transfer to liquid nitrogen tank or -140°C.

5. Performance Assay

This section outlines the recommended procedure to measure the performance of the GloResponse™ NF-κB-RE-*luc2P* HEK293 Cell Line in response to TNFα in 384- or 96-well assay formats. Volumes should be scaled appropriately for different plates and the desired number of replicate samples for each condition. Dispense solutions with a multichannel pipettor whenever possible.

5.A. Cell Culture Preparation

Two or three days before performing the assay, split cells in growth medium so that they will be 100% confluent the day before the assay. Replace the medium in the flasks with Growth Medium (no hygromycin B) the day before the assay.

5.B. Recommended Control and Treatment Conditions

Treatment or Control	Composition (per well in a 384-well plate)	Purpose
Unstimulated Control	Cells in 20μl Assay Medium (Section 3.B) and 5μl additional Assay Medium	Measures uninduced level of NF-κB reporter activity.
TNFα-Stimulated	Cells in 20μl Assay Medium (Section 3.B) and 5μl 5X TNFα solution (Section 3.B)	Measures induced level of NF-κB reporter activity.
Cell-Free Control	25μl Assay Medium (Section 3.B)	Determines background luminescence of instrument.

5.C. Preparation of Induction and Control Solutions

1. Prepare 5X TNF α solution in Assay Medium (100ng/ml TNF α). The final concentration in the well will be 20ng/ml TNF α .

5.D. Performance Assay Protocol

(Protocol is for 384-well format; volumes for 96-well format are provided in parentheses.)

1. For a 384-well plate, add 5 μ l of Assay Medium to the Unstimulated Control wells (20 μ l for a 96-well plate).
2. Add 5 μ l of 5X TNF α solution to TNF α -Stimulated wells (20 μ l for a 96-well plate).
3. Add 25 μ l of Assay Medium to Cell-Free Control wells (100 μ l for a 96-well plate). Return the plate to the tissue culture incubator while preparing cells in Step 4.

4. Harvest as described in Section 4.B, Steps 1–8, using Assay Medium. Resuspend cells in Assay Medium to a density of 5.0×10^5 cells/ml.

Note: GloResponse™ NF- κ B-RE-*luc2P* HEK293 cells tend to clump. To fully resuspend cells, pipet prior to plating.

5. Add 20 μ l/well of cell suspension to Unstimulated Control wells and TNF α -Stimulated wells (80 μ l for a 96-well plate).
6. Incubate the assay plate in a humidified 37°C, 5% CO₂ incubator for 5 hours.
7. Reconstitute reagents as directed in the *ONE-Glo™ Luciferase Assay System Technical Manual (#TM292)* to measure firefly luciferase activity.

Note: If a doubly transfected, stable cell line expressing a target receptor of interest has been generated using the pF9A CMV *hRluc*-neo Flexi® Vector (Cat.# C9361) and both firefly luciferase and *Renilla* luciferase are to be read from the same sample, then use the Dual-Glo® Luciferase Assay System Reagent to measure luciferase activity.

8. Add 25 μ l of ONE-Glo™ Luciferase Assay System Reagent to each well (100 μ l for a 96-well plate).
9. Incubate at room temperature for at least 3 minutes.
10. Measure luminescence using a luminometer; read for 0.5 second/well.

5.E. Data Analysis

The formula to calculate reporter gene induction by TNF α is provided below.

$$\text{induction} = \frac{(\text{average stimulated wells} - \text{average Cell-Free Control wells})}{(\text{average Unstimulated Control wells} - \text{average Cell-Free Control wells})}$$

5.F. Sample Data

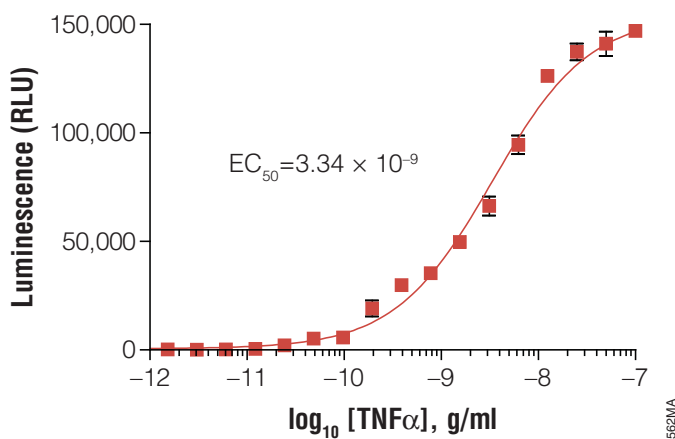


Figure 1. GloResponse™ NF-κB-RE-luc2P HEK293 cells response to TNFα titration.

A total of 10,000 GloResponse™ NF-κB-RE-luc2P HEK293 cells per well were dispensed into each well of a 384-well plate, and twofold serial dilutions of TNFα were added to induce reporter gene expression. After 5 hours of induction in a tissue culture incubator, luciferase activity was quantified using the ONE-Glo™ Luciferase Assay System Reagent on the Berthold® LB 96 V Luminometer. n = 8 for each data point.

6. Appendix

6.A. References

1. Brasier, A.R. and Ron, D. (1992) Luciferase reporter gene assay in mammalian cells. *Methods Enzymol.* **216**, 386-97.
2. Zhuang, F. and Liu, Y.H. (2006) Usefulness of the luciferase reporter system to test the efficacy of siRNA. *Methods Mol. Biol.* **342**, 181-7.
3. Paguio, A. *et al.* (2005) pGL4 Vectors: A new generation of luciferase reporter vectors. *Promega Notes* **89**, 7-10.

6.B. Related Products

Product	Size	Cat.#
GloResponse™ CRE- <i>luc</i> 2P HEK293 Cell Line	2 × 10 ⁶ cells	E8500
GloResponse™ NFAT-RE- <i>luc</i> 2P HEK293 Cell Line	2 × 10 ⁶ cells	E8510
ONE-Glo™ Luciferase Assay System	10ml*	E6110
Dual-Glo® Luciferase Assay System	10ml*	E2920
Bright-Glo™ Luciferase Assay System	10ml*	E2610
Steady-Glo® Luciferase Assay System	10ml*	E2510
GloMax®-Multi Detection System	1 each	E7031
GloMax® 96 Microplate Luminometer	1 each	E6501
pGL4.32[<i>luc</i> 2P/NF-κB-RE/Hygro] Vector	20μg	E8491
pF9A CMV <i>hRluc</i> -neo Flexi® Vector	20μg	C9361

*Additional Sizes Available.

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©European Pat. No. 1341808 and other patents pending.

©Patent Pending.

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