



Promega

Technical Bulletin

GloResponse™ 9XGAL4UAS-*luc2P* HEK293 Cell Line

INSTRUCTIONS FOR USE OF PRODUCT E8530.



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GloResponse™ 9XGAL4UAS-*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™ 9XGAL4UAS-*luc2P* HEK293 Cell Line^(a-h) is a clonal derivative of Human Embryonic Kidney 293 (HEK293)^(e) cells. These cells contain nine repeats of GAL4 UAS (Upstream Activator Sequence). This sequence drives the transcription of the luciferase reporter gene *luc2P* in response to binding of a fusion protein containing the Gal4 DNA Binding Domain (e.g., the Estrogen Receptor Ligand Binding Domain in pBIND-ER α Vector [Cat.# E1390]) when activated by a ligand.

The GloResponse™ 9XGAL4UAS-*luc2P* HEK293 Cell Line was generated by clonal selection of HEK293 cells stably transfected with the pGL4.35[*luc2P*/9XGAL4UAS/Hygro] Vector (Cat.# E1370). 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

for improved 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™ 9XGAL4UAS- <i>luc2P</i> HEK293 Cell Line	2 vials	E8530

Includes: Two vials of approximately 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 in U.S.; Cat.# 41965-03 in U.K.)
- DMEM without phenol red (Invitrogen Cat.# 21063-029)
- defined fetal bovine serum (FBS; Hyclone Cat.# SH30071)
- charcoal/dextran-treated FBS (Hyclone Cat.# SH30068.02)
- 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)
- TrypLE™ Express Stable Trypsin without phenol red (Invitrogen Cat.# 12604-013; for use in performance assay)
- Promega luciferase assay reagent (see Section 6.B., Related Products)
- pBIND-ER α Vector (Promega Cat.# E1390) or pBIND-GR Vector (Promega Cat.# E1581)
- β -estradiol (Sigma-Aldrich Cat.# E2758) or dexamethasone (Sigma-Aldrich Cat.# D4902)
- transfection reagent (e.g., Mirus Bio MIR 2304 or similar product)

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
- 200µg/ml hygromycin B

Freezing Medium

- 80% DMEM
- 15% FBS
- 5% DMSO

Assay Medium

- 95% DMEM without phenol red
- 5% charcoal/dextran-treated FBS

β-Estradiol (E2) Stock

- 100µM in ethanol

dexamethasone stock

- 10mM in ethanol

4. Maintenance of GloResponse™ 9XGAL4UAS-*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 x g for 5 minutes at 18°C (or below room temperature).
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 cells one time 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 $500 \times g$ for 5 minutes at 18°C (or below room temperature).
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 in 10–12ml 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 for measuring performance of the GloResponse™ 9XGAL4UAS-*luc2P* HEK293 Cell Line in response to induction of the ER-ligand binding domain with β -estradiol or GR-ligand binding domain with dexamethasone in 96- or 384-well assay formats. Volumes should be scaled appropriately for different density plates and for the desired number of replicate samples for each condition. Dispense solutions with a multichannel pipettor whenever possible.

5.A. Performance Assay Protocol

Table 1. Recommended Timeline for Performance Assay Protocol.

Treatment or Control	96-Well Assay	384-Well Assay
Day 1	Seed cells in 96-well plate.	Seed cells in 10cm dish.
Day 2	Transfect cells with pBIND-ER α or pBIND-GR Vector.	Transfect cells with pBIND-ER α or pBIND-GR Vector.
Day 3	Induce cells.	Transfer cells to 384-well plate and induce.
Day 4	Read luminescence.	Read luminescence.

Protocol in Detail

Day 1: Plate Cells

For 96-well plate: Seed HEK293 cells at 10,000 cells/well in a solid white 96-well tissue culture plate using phenol red-free DMEM containing 5% charcoal/dextran-treated FBS (80 μ l/well).

For 384-well plates: Seed 2×10^6 cells in a 10cm dish.

Note: Use trypsin without phenol red to dissociate cells, or pellet and wash cells twice with PBS to remove phenol red.

Day 2: Transfect Cells

1. Transfect the cells using a high-efficiency transfection reagent. Each well of the 96-well plate to be transfected requires 100ng of a fusion gene containing the Gal4 DNA Binding Domain such as pBIND-ER α Vector (Cat.# E1390) or pBIND-GR Vector (Cat.# E1581). Transfection conditions may require optimization. We have routinely added approximately 10 μ l/well of a transfection master mix.
2. Cover the plate and place it in a tissue culture incubator at 37°C overnight or as needed for cell recovery, depending on the transfection method used. We use 24 hours for recovery time for lipid-mediated transfections.

For 384-well plates: Transfect cells in a 10cm dish.

Day 3: Induce Transfected Cells

1. Prepare 10X induction and 10X control solution. Calculate the volume of 10X induction and 10X control solution to prepare by multiplying the number of wells using each solution by 10 μ l, then prepare at least 110% of this amount.

Table 2. Induction Conditions Used with the pBIND-ER α and pBIND-GR Vector DNA.

Vector	Induction Reagent	Stock Solution (in ethanol)	10X Induction Solution (10 μ l \times n wells \times 1.1)	Final Conc. per Well	10X Control Solution (10 μ l \times n wells \times 1.1)
pBIND-GR	Dex	10mM	100 μ M	10 μ M	1% ethanol
pBIND-ER α	E2	100 μ M	100nM	10nM	0.1% ethanol

- **10X Induction Solution:** For induction with pBIND-GR Vector, dilute 10mM dexamethasone solution in DMEM without phenol red to 100 μ M (1:100 dilution). Final dexamethasone concentration will be 10 μ M. For induction with pBIND-ER α Vector, dilute 100 μ M E2 stock solution with DMEM without phenol red to 100nM (1:1,000 dilution). Final E2 concentration will be 10nM.

- **Control Solution:** To control for both induction reagents, add the same volume of ethanol as was used in 10X induction solution (see Table 2) to DMEM without phenol red.

2. Add 10 μ l of 10X induction solution to wells to be induced or add control solution to control, noninduced wells.

For 384-well plates: On day 3, trypsinize cells and transfer to 384-well solid white plate at 10,000 cells/well in a volume of 20 μ l. Induce cells by adding 5 μ l/well of a 5X induction solution (50 μ M dex or 50nM E2) or a 5X control solution (0.5% ethanol for dex control or 0.05% ethanol for E2 control).

Day 4: Read Luminescence

Analyze luciferase activity using an appropriate luciferase detection assay system (from Promega; see Section 6.B., Related Products).

For 384-well plates: Read luminescence.

5.B. Data Analysis

Use this formula to calculate fold induction:

$$\text{Fold induction} = \frac{\text{Average relative light units of induced cells}}{\text{Average relative light units of control cells}}$$

5.C. Sample Data

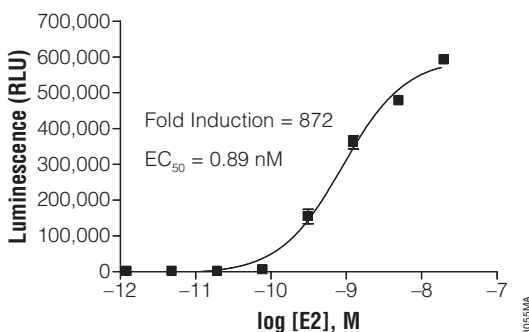


Figure 1. E2 titration of GloResponse™ 9XGAL4UAS-*luc2P* HEK293 cells transfected with pBIND-ER α Vector. A total of 10,000 transfected GloResponse™ 9XGAL4UAS-*luc2P* HEK293 cells per well were dispensed into each well of a 384-well plate, and four-fold serial dilutions of E2 were added to induce reporter gene expression. After a 24-hour induction in a tissue culture incubator, luciferase activity was quantified using the Dual-Glo® Luciferase Assay System Reagent on the Thermo VarioSkan Luminometer. n = 4 for each data point.

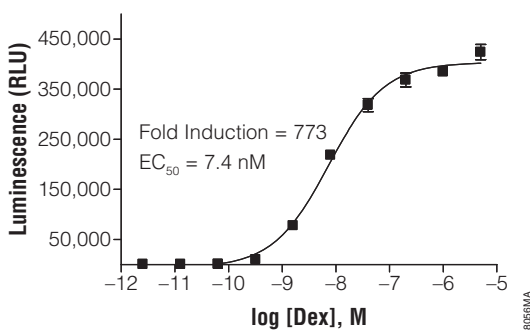


Figure 2. Dexamethasone titration of GloResponse™ 9XGAL4UAS-*luc2P* HEK293 cells transfected with pBIND-GR Vector. A total of 10,000 GloResponse™ 9XGAL4UAS-*luc2P* HEK293 cells were dispensed into each well of a 384-well plate, and five-fold serial dilutions of dexamethasone added to induce reporter gene expression. After a 24-hour induction in a tissue culture incubator, luciferase activity was quantified using the Dual-Glo® Luciferase Assay System Reagent on the Thermo VarioSkan Luminometer. n = 4 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
GloResponse™ NF-κB-RE- <i>luc</i> 2P HEK293 Cell Line	2 × 10 ⁶ cells	E8520
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
pGL4.35[<i>luc</i> 2P/9XGAL4UAS/Hygro] Vector	20µg	E1370
pFN26A (BIND) <i>hRluc</i> -neo Flexi® Vector	20µg	E1380
pBIND-ERα Vector	20µg	E1390
pBIND-GR Vector	20µg	E1581

†For Laboratory Use.

*Additional Sizes Available.

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Ⓑ U.S. Pat. No. 5,670,356.

Ⓒ Australian Pat. No. 2003272419 and other patents pending.

Ⓓ Australian Pat. No. 2001285278 and other patents pending.

Ⓔ HEK293 cells were obtained under license from AdVec Inc.

Ⓕ Commercial use of this cell line requires a license from AdVec Inc.

Ⓖ The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

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