

# GloResponse™ 9XGAL4UAS-*luc2P* HEK293 Cell Line

INSTRUCTIONS FOR USE OF PRODUCT E8530.

Quick  
PROTOCOL

## Getting Started

Section 3 of the *GloResponse™ 9XGAL4UAS-luc2P HEK293 Cell Line Technical Bulletin*, #TB552 contains lists of recommended reagents, supplies and equipment, plus recipes for required media and buffers.

Section 4 of TB552 has information on how to thaw and culture the GloResponse™ cell line, as well as a procedure for freezing the cells.

For complete protocol and ordering information see TB552, available online at:  
[www.promega.com/tbs/tb552/tb552.html](http://www.promega.com/tbs/tb552/tb552.html)

## Performance Assay Protocol

### Day 1: Plate Cells

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

**For 384-well plates:** Seed  $2 \times 10^6$  cells in a 10cm dish.

### 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 the pBIND-ER $\alpha$  Vector (Cat.# E1390) or the pBIND-GR Vector (Cat.# E1581). Transfection conditions may require optimization. We routinely add approximately 10µl/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 a recovery time of 24 hours for lipid-mediated transfections.

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

### Day 3: Induce Transfected Cells (see Table, next page)

1. Prepare 10X induction and 10X control solutions. Calculate the volume of 10X induction and 10X control solution needed by multiplying the number of wells using each solution by 10µl. Prepare at least 110% of this amount.
  - **10X Induction Solution:** For induction with the pBIND-GR Vector, dilute 10mM dexamethasone solution in DMEM without phenol red to 100µM (1:100 dilution). Final dexamethasone concentration will be 10µM. For induction with the pBIND-ER $\alpha$  Vector, dilute 100µM E2 stock solution with DMEM without phenol red to 100nM (1:1,000 dilution). Final E2 concentration will be 10nM.
  - **Control Solution:** Add the same volume of ethanol used in 10X induction solution to DMEM without phenol red (see table, next page).
2. Add 10µl of 10X induction solution to wells to be induced or add control solution to 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 20µl. Induce cells by adding 5µl/well of a 5X induction solution (50µM dex or 50nM E2) or a 5X control solution (0.5% ethanol for dex control or 0.05% ethanol for E2 control).

### ORDERING/TECHNICAL INFORMATION:

[www.promega.com](http://www.promega.com) • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

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## Performance Assay Protocol (continued)

### Day 4: Read Luminescence

For **96-well and 384-well plates**: Analyze luciferase activity using an appropriate Promega luciferase detection assay system (for available products see Section 6.B, Related Products, TB552).

### Induction Conditions Used with the pBIND-ER $\alpha$ and pBIND-GR Vector DNA.

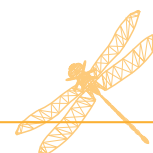
Vector	Induction Reagent	Stock Solution (in ethanol)	10X Induction Solution (10 $\mu$ l $\times$ n wells $\times$ 1.1)	Final Conc. per Well	10X Control Solution (10 $\mu$ l $\times$ n wells $\times$ 1.1)
pBIND-GR	Dex	10mM	100 $\mu$ M	10 $\mu$ M	1% ethanol
pBIND-ER $\alpha$	E2	100 $\mu$ M	100nM	10nM	0.1%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

For the complete protocol visit: [www.promega.com/tbs/tb552/tb552.html](http://www.promega.com/tbs/tb552/tb552.html)

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