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- Trockeneiszuschlag
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Data Sheet

GAL4 Reporter Kit (*Glucocorticoid Receptor Pathway)*
Catalog #: 60522

Background
The glucocorticoid signaling pathway plays an important role in development, fluid homeostasis, cognition, immune response and metabolism. Glucocorticoids are a class of steroid hormones that bind to the glucocorticoid receptor, causing it to translocate to the nucleus. Upon translocation, the receptor can regulate the transcription of a large number of genes, including those that regulate glucose metabolism and inflammatory responses.

Description
The Glucocorticoid Receptor Pathway Reporter Kit is designed for monitoring the activity of the glucocorticoid signaling pathway in cultured cells. The kit contains a transfection-ready expression vector for the glucocorticoid receptor ligand binding domain that is fused to the DNA binding domain (DBD) of GAL4 (GAL4 DBD-GR). This fusion construct activates firefly luciferase expression under the control of a multimerized GAL4 upstream activation sequence (UAS). This allows for specific detection of glucocorticoid-induced activation of the glucocorticoid receptor without the need for individual transcriptional targets and with low cross-reactivity for other nuclear receptor pathways. The GAL4/UAS reporter is premixed with constitutively expressing *Renilla* (sea pansy) luciferase vector, which serves as an internal positive control for transfection efficiency.

The kit also includes a non-inducible firefly luciferase vector premixed with constitutively-expressing *Renilla* luciferase vector as a negative control. The non-inducible luciferase vector contains a firefly luciferase gene under the control of a minimal promoter, but without any additional response elements. The negative control is critical for determining pathway-specific effects and background luciferase activity.

Application
- Monitor glucocorticoid signaling pathway activity.
- Screen activators or inhibitors of the glucocorticoid signaling pathway.

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Components

Component	Specification	Amount	Storage
Reporter (Component A)	GAL4/UAS luciferase reporter vector + constitutively expressing *Renilla* luciferase vector	500 µl (60 ng DNA/µl)	-20°C
Negative Control Reporter (Component B)	Non-inducible luciferase vector + constitutively expressing *Renilla* luciferase vector	500 µl (60 ng DNA/µl)	-20°C
GAL4 DBD-GR (Component C)	Expression vector for ligand binding domain of the glucocorticoid receptor + GAL4 DNA binding domain	250 µl (100 ng DNA/µl)	-20°C
Negative Control Expression vector (Component D)	Expression vector with GAL4 DNA binding domain only	250 µl (100 ng DNA/µl)	-20°C

These vectors are ready for transient transfection. They are NOT SUITABLE for transformation and amplification in bacteria.

Materials Required but Not Supplied

- Mammalian cell line and appropriate cell culture medium
- 96-well tissue culture plate or 96-well tissue culture treated white clear-bottom assay plate (Corning # 3610)
- Transfection reagent for mammalian cell line. [We use Lipofectamine™ 2000 (Life Technologies #11668027), however, other transfection reagents should work equally well.]
- Opti-MEM I Reduced Serum Medium (Life Technologies #31985-062)
- Dual luciferase assay system:
  Dual Luciferase (Firefly-Renilla) Assay System (BPS Cat. #60683): This system assays cells directly in growth medium. It can be used with any luminometer. Automated injectors are not required.
- Luminometer

Generalized Transfection and Assay Protocols

The following procedure is designed to transfect the reporter into HEK293 cells using Lipofectamine 2000 in a 96-well format. To transfect cells in different tissue culture formats, adjust the amounts of reagents and cell number in proportion to the relative surface area. If using a transfection reagent other than Lipofectamine 2000, follow the manufacturer's transfection protocol. Transfection condition should be optimized according to the cell type and study requirements.
All amounts and volumes in the following setup are given on a per well basis.

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1. One day before transfection, seed cells at a density of ~30,000 cells per well in 100 µl of growth medium so that cells will be 90% confluent at the time of transfection.

2. The next day, for each well, prepare complexes as follows:

   a. Dilute DNA mixtures in 15 µl of Opti-MEM I medium (antibiotic-free). Mix gently. Depending upon the experimental design, the DNA mixtures may be any of the following combinations:
      - 1 µl of **Reporter** (component A); in this experiment, the control transfection is 1 µl of **Negative Control Reporter** (component B).
      - 1 µl of **Reporter** (component A) + **experimental vector** expressing gene of interest (such as component C); in this experiment, the control transfections are: 1 µl of **Reporter** (component A) + **negative control expression vector** (such as component D), 1 µl of **Negative Control Reporter** (component B) + **experimental vector** expressing gene of interest, and 1 µl of **Reporter** (component A) + **negative control expression vector** (component D).
      - 1 µl of **Reporter** (component A) + **specific siRNA**; in this experiment, the control transfections are: 1 µl of **Reporte**r (component A) + **negative control siRNA**, 1 µl of **Negative Control Reporter** (component B) + **specific siRNA**, and 1 µl of **Negative Control Reporter** (component B) + **negative control siRNA**.

Note: we recommend setting up each condition in at least triplicate, and preparing transfection cocktail for multiple wells.

   b. Mix Lipofectamine 2000 gently before use, then dilute 0.35 µl of Lipofectamine 2000 in 15 µl of Opti-MEM I medium (antibiotic-free). Incubate for 5 minutes at room temperature. Note: Prepare this dilution cocktail in volumes sufficient for the whole experiment.

   c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine 2000. Mix gently and incubate for 25 minutes at room temperature.

3. Add the 30 µl of complexes to each well containing cells and medium. Mix gently by tapping the plate.

4. Incubate cells at 37°C in a 5% CO_2 incubator. After ~24 hours of transfection, change medium to fresh growth medium. ~48 hours after transfection, perform the Dual Luciferase Assay System following the protocol on the BPS data sheet (BPS Cat. #60683).

To study the effect of activators/inhibitors on the glucocorticoid pathway, treat the cells with tested activator/inhibitor after ~6 hours or 24 hours of transfection. Perform dual luciferase assay ~48 hours after transfection.

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Sample protocol to determine the effect of Dexamethasone on GAL4 DBD-GR combined with the GAL4/UAS reporter in HEK293 cells

Additional materials required in this experimental setup

- Dexamethasone
- HEK293 assay medium: DMEM (without phenol red) (Hyclone #SH30604.01) + 10% charcoal/dextran-treated FBS (Hyclone #SH30068.02), 4mM L-Glutamine + 1% Pen/Strep
- 96-well tissue culture treated white clear-bottom assay plate (Corning #3610)
- Dual Luciferase (Firefly-Renilla) Assay System (BPS Cat. #60683)

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well into white clear-bottom 96-well plate in 100 µl of assay medium (DMEM without phenol red, 10% charcoal/dextran-treated FBS, 4 mM L-Glutamine, 1% Pen/Strep). Incubate cells at 37°C in a CO_2 incubator overnight.

2. The next day, transfect 1 µl of GAL4/UAS luciferase reporter (component A) or 1 µl negative control reporter (component B) with 0.5 µl of GAL4 DBD-GR (component C) or negative control expression vector (component D) into cells following the procedure in **Generalized Transfection and Assay Protocols.**

3. After ~24 hours of transfection, treat transfected cells with 3 µM or various concentrations of Dexamethasone (if determining response curve) in 50 µl of fresh assay medium. Add 50 µl of assay medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate. Incubate cells at 37°C in a CO_2 incubator for ~24 hours.

4. After ~48 hours of transfection, perform dual luciferase assay using BPS Dual Luciferase (Firefly-Renilla) Assay System (BPS Cat. #60683): Dilute 100x Firefly Luciferase Reagent Substrate (Component B) into Firefly Luciferase Reagent Buffer (Component A). Add 50 µl of Firefly Luciferase reagent per well and rock at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Dilute 100x Renilla Luciferase Reagent Substrate (Component D) into Renilla Luciferase Reagent Buffer (Component C). Add 50 µl of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute and measure Renilla luminescence.

5. To obtain the normalized luciferase activity for GAL4/UAS reporter, subtract background luminescence, then calculate the ratio of firefly luminescence from GAL4/UAS reporter to *Renilla* luminescence from the control *Renilla* luciferase vector.

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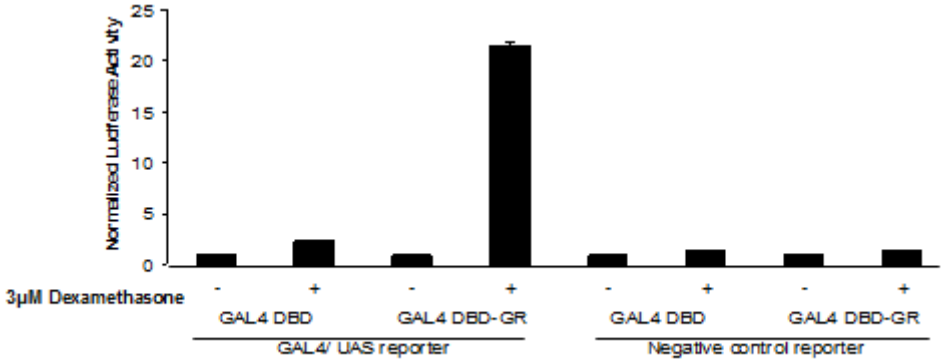
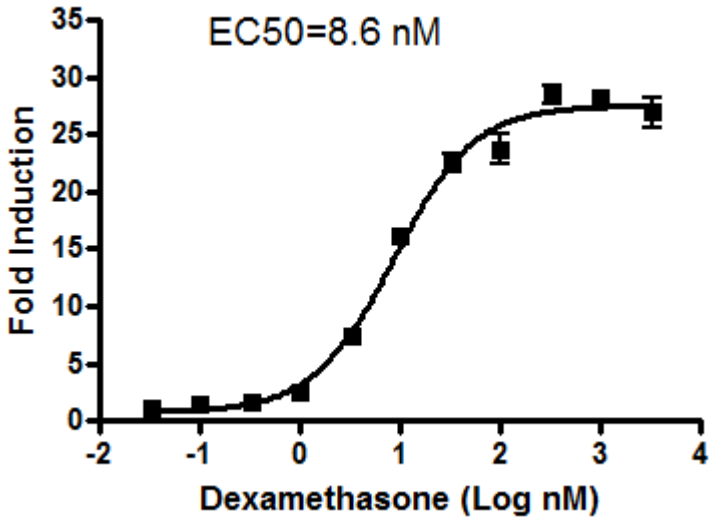
Figure 1. (A) Dexamethasone-induced activation of the GAL4/UAS reporter with the GAL4 DBD-GR expression vector. The results are shown as normalized GAL4/UAS luciferase reporter activity.



(B) Dose response of GAL4 DBD-GR expression vector with GAL4/UAS reporter activity to Dexamethasone. The results are shown as fold induction of normalized GAL4/UAS luciferase reporter activity. Fold induction was determined by comparing values against the mean value for control cells without Dexamethasone treatment.



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Sample protocol to determine the effect of antagonists of the Glucocorticoid signaling pathway on Dexamethasone-induced GAL4 DBD-GR with GAL4/UAS reporter activity in HEK293 cells:

Additional materials required in this experiment setup

- Dexamethasone
- Mifepristone/ RU-486 (Sigma #M8046)
- HEK293 assay medium: DMEM (without phenol red) (Hyclone #SH30604.01) + 10% charcoal/dextran treated FBS (Hyclone #SH30068.02), 4 mM L-Glutamine + 1% Pen/Strep
- 96-well tissue culture treated white clear-bottom assay plate (Corning #3610)
- Dual Luciferase (Firefly-Renilla) Assay System (BPS Cat. #60683)

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells in 100 µl of assay medium into each well of a white clear-bottom 96-well plate. Incubate cells at 37°C in a 5% CO_2 incubator overnight.

2. The next day, transfect 1 µl of GAL4/UAS luciferase reporter (component A) with 0.5 µl of GAL4 DBD-GR (component C) or negative control expression vector (component D) into cells following the procedure in **Generalized Transfection and Assay Protocols.**

3. After ~24 hours of transfection, treat transfected cells with 100 nM (final concentration) of the glucocorticoid pathway antagonist Mifepristone in 45 µl of fresh assay medium. Set up each treatment in at least triplicate. Include a subset of untreated, control wells that do not receive Mifepristone.

4. Incubate cells at 37°C in a CO_2 incubator for ~1 hour.

5. Add Dexamethasone (final concentration 10 nM) in 5 µl of assay medium to stimulated wells (cells treated with Dexamethasone, with or without Mifepristone); add 5 µl of assay medium to the unstimulated control wells (for determining the basal activity); add 50 µl of growth medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate.

6. Incubate cells at 37°C in a CO_2 incubator for ~24 hours.

7. Perform dual luciferase assay using BPS Dual Luciferase (Firefly-Renilla) Assay System (BPS Cat. #60683): Dilute 100x Firefly Luciferase Reagent Substrate (Component B) into Firefly Luciferase Reagent Buffer (Component A). Add 50 µl of Firefly Luciferase reagent per well and rock at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Dilute 100x Renilla Luciferase Reagent Substrate (Component D) into Renilla Luciferase Reagent Buffer (Component C). Add 50 µl of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute and measure Renilla luminescence.

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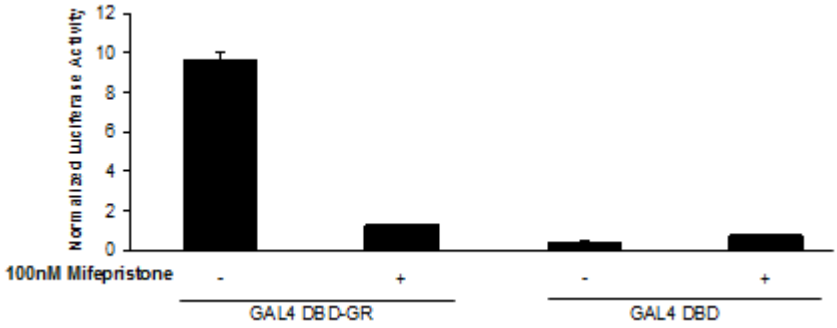
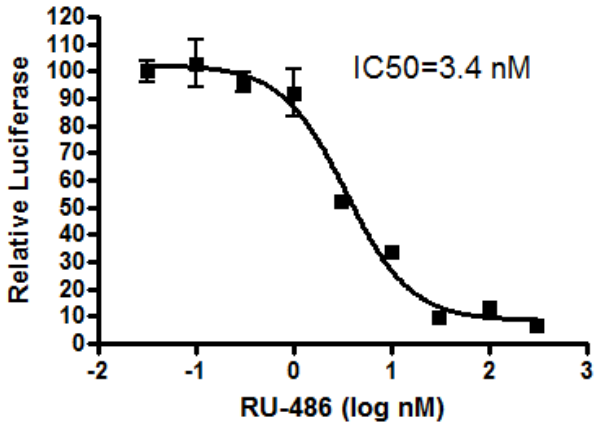
5. To obtain the normalized luciferase activity for GAL4/UAS reporter, subtract background luminescence, then calculate the ratio of firefly luminescence from GAL4/UAS reporter to *Renilla* luminescence from the control *Renilla* luciferase vector.

Figure 2. Inhibition of Dexamethasone-induced GAL4 DBD-GR with GAL4/UAS reporter activity by the Glucocorticoid antagonist, Mifepristone. (A) Mifepristone completely blocks Dexamethasone-induced GAL4 DBD-GR with GAL4/UAS reporter activity. The results are shown as normalized GAL4/UAS luciferase reporter activity.



(B) Dose response of inhibition of Dexamethasone-induced GAL4 DBD-GR with GAL/UAS reporter activity to Mifepristone. The results are shown as percentage of GAL4/UAS reporter activity. The normalized luciferase activity for GAL4 DBD-GR transfected cells treated with 10 nM Dexamethasone without Mifepristone treatment was set at 100%.



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Reference

Paguio A, *et al*. (2010) Improved Dual-Luciferase Reporter Assays for Nuclear Receptors. *Curr Chem Genomics*. **4:** 43-49.

Related Products

Product	Cat. #	Size
AP-1 Reporter Kit	60612	500 rxns.
NF-κB Reporter Kit	60614	500 rxns.
Dual Luciferase (Firefly-Renilla) Assay System	60683-1	10 mL
Dual Luciferase (Firefly-Renilla) Assay System	60683-2	100 mL
Dual Luciferase (Firefly-Renilla) Assay System	60683-3	1 L
ARE Reporter Kit	60514	500 rxns.
Myc Reporter Kit	60519	500 rxns.
Notch1/CSL Reporter Kit	60509	500 rxns.
AP-1 Reporter – HEK293 Cell Line	60405	2 vials
NF-κB Reporter – HEK293 Cell Line	60650	2 vials
ARE Reporter – HepG2 Cell Line	60513	2 vials
Notch1/CSL Reporter - HEK293 Cell Line	60652	2 vials
RARα Reporter Cell Line	60503	2 vials
RARβ Reporter Cell Line	60603	2 vials
RARγ Reporter Cell Line	60604	2 vials