



User Manual

Signal[™] Pathway Reporter Kits

Cell-Based Pathway Activity Assays

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Part # 1030A

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Signal™ Reporter Assay Kits

Cell-Based Pathway Activity Assays

User Manual

(For Catalog Numbers: 336841 (CCS-###L and CCS-###G))

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I. Introduction

The Signal Reporter Assays are designed for accurate, sensitive and quantitative assessment of the activation of signal transduction pathways. We have developed a series of inducible reporter constructs that encode a reporter gene under the control of a basal promoter element (TATA box) joined to tandem repeats of specific transcriptional response elements (TRE; Figure 1). Transcription factor activities can act as readouts for the intracellular status of many signal transduction pathways. Our constructs are specifically engineered for measuring changes in activity (both increases and decreases) of these signaling pathways. Each of the Signal reporter assays is available in a dual-luciferase format. In addition, sixteen of the Signal reporter assays are also available as GFP reporter constructs. The Signal reporter assays are valuable tools for deciphering gene function, as well as determining the mechanism of action of proteins, peptides, ligands, and small molecule compounds.

Each of the dual-luciferase assays has a pathway-specific firefly reporter and a *Renilla* luciferase normalization reporter. The pathway-specific reporter encodes a mammalian codon-optimized, non-secreted form of the firefly luciferase gene, carrying a protein-destabilizing sequence. Cells rapidly degrade the destabilized form of the firefly luciferase protein and hence the background luciferase activity is reduced. Due to lower background activity, the magnitude of the response that can be measured (signal-to-noise ratio) as well as the speed of measuring changes in transcription are enhanced. The *Renilla* luciferase reporter is constitutively expressed. The Signal dual-luciferase reporter assays provide outstanding reproducibility, sensitivity, specificity, and signal-to-noise ratio.

The Signal GFP reporter assays monitor the dynamics of pathway activation in living cells with single cell resolution. The Signal GFP reporter constructs encode an improved synthetic version of the green fluorescent protein gene, Monster Green™ Fluorescent Protein. Monster GFP has been codon optimized and has an optimized Kozak sequence to maximize mammalian cell expression. The synthetic GFP is an ideal fluorescent reporter, providing high-level fluorescence and minimal cytotoxicity. Moreover, the synthetic GFP gene is resistant to photobleaching. The spectral properties of the synthetic GFP are slightly red-shifted compared to other commercially available GFPs. Peak excitation occurs at 505nm, with a shoulder at 480nm; peak emission occurs at 515nm.

Benefits of Signal Reporter Assays

- **PERFORMANCE:** Dual-luciferase and GFP reporter systems provide exceptional sensitivity, reproducibility, specificity, and signal-to-noise ratio
- **VERSATILITY:** Monitor pathway activity with a luminescent or fluorescent reporter assay
- **CONVENIENCE:** Transfection-ready, Positive and Negative controls included

HOW SIGNAL REPORTER (LUC) ASSAYS WORK

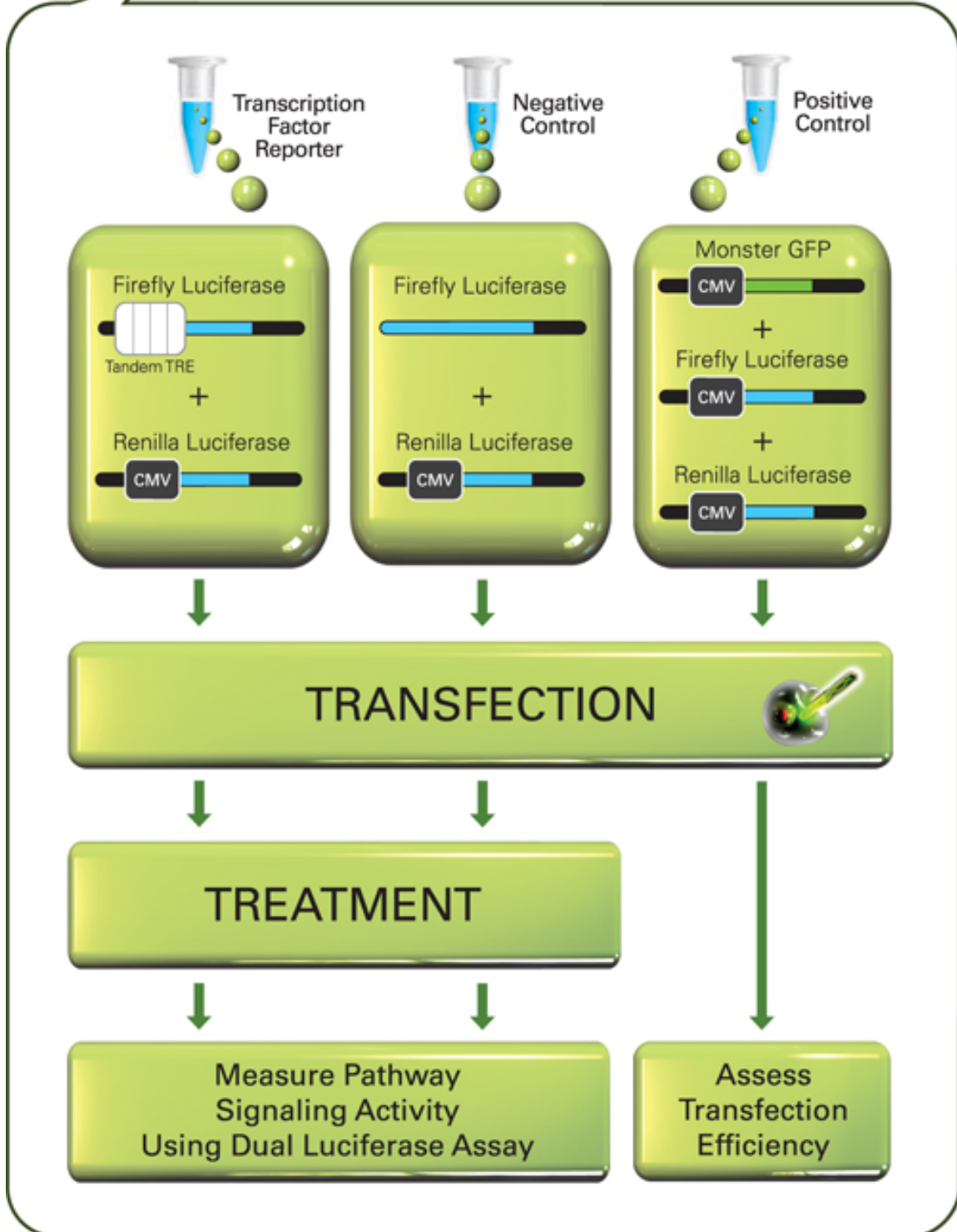


Figure 1A: Overview of Signal™ Dual-Luciferase Reporter Assays Process.

HOW SIGNAL REPORTER (GFP) ASSAYS WORK

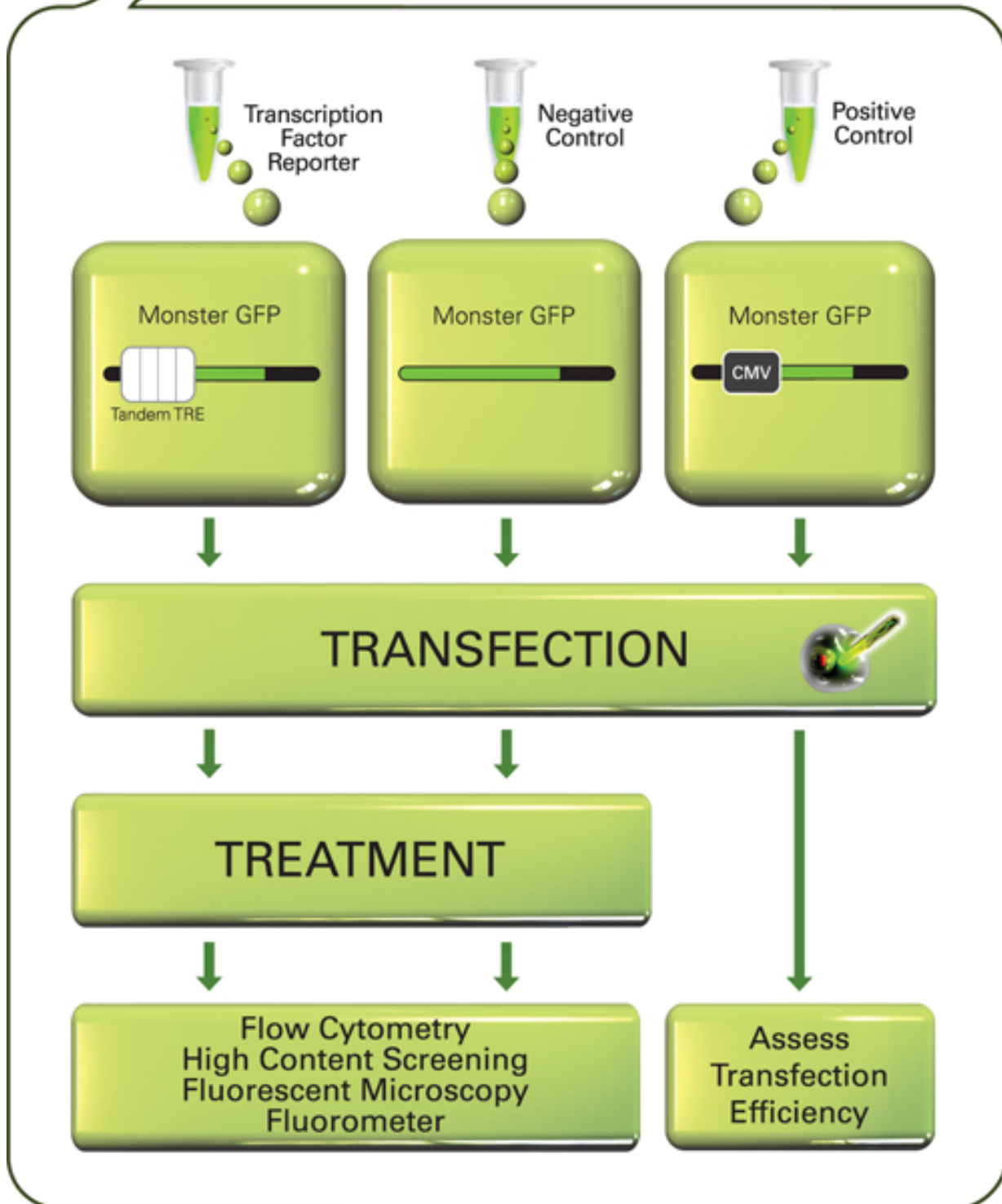


Figure 1B: Overview of Cignal™ GFP Reporter Assays Process.

II. Product Contents and Descriptions

A. Dual-Luciferase Reporter Assay Kits:

1. Kit Contents

Table 1: Signal Reporter Assay Kit Specifications

Component	Specification	Concentration ; Volume
Reporter	A mixture of an inducible transcription factor responsive firefly luciferase reporter and constitutively expressing <i>Renilla</i> construct (40:1).	(100 ng/ μ l; 500 μ l)
Negative control	A mixture of non-inducible firefly luciferase reporter and constitutively expressing <i>Renilla</i> construct (40:1).	(100 ng/ μ l; 500 μ l)
Positive control	A mixture of a constitutively expressing GFP construct, constitutively expressing firefly luciferase construct, and constitutively expressing <i>Renilla</i> luciferase construct (40:1:1).	(100 ng/ μ l; 250 μ l)

NOTE: *These constructs are **transfection-grade** and are ready for transient transfection. These constructs are specifically designed to inhibit transformation and are NOT MEANT for introduction and amplification in bacteria.*

2. Description:

Each Signal Reporter Assay Kit includes the following components:

- Reporter:** The reporter is a mixture of inducible transcription factor responsive construct and constitutively expressing *Renilla* luciferase construct (40:1). The inducible transcription factor-responsive construct encodes the firefly luciferase reporter gene under the control of a basal promoter element (TATA box) joined to tandem repeats of a specific Transcriptional Response Element (TRE; Figure 2A). This construct monitors both increases and decreases in the activity of a key transcription factor, which is a downstream target of a specific signaling pathway. The constitutively expressing *Renilla* construct encodes the *Renilla* luciferase reporter gene under the control of a CMV immediate early enhancer/promoter (Figure 2B) and acts as an internal control for normalizing transfection efficiencies and monitoring cell viability. It is also useful to confirm transfection and to verify active luciferase in the transfected culture.
- Negative control:** The negative control is a mixture of non-inducible reporter construct and constitutively expressing *Renilla* luciferase construct (40:1). The non-inducible reporter construct encodes firefly luciferase under the control of a basal promoter

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element (TATA box), without any additional transcriptional response elements (Figure 2C). The negative control is critical to identifying non-specific effects and determining background reporter activity.

3. **Positive control:** The positive control is a constitutively expressing GFP construct (Figure 2D), pre-mixed with a constitutively expressing firefly luciferase construct (Figure 2E), and a constitutively expressing *Renilla* luciferase construct (Figure 2B) (40:1:1). The positive control is necessary for visual confirmation of transfection. It is also useful for transfection optimization studies. The expression of the GFP from the positive control construct can be monitored by fluorescence microscopy using an excitation filter of 470 ± 20 nm (470 / 40 nm) and an emission filter of 515 nm (long pass).

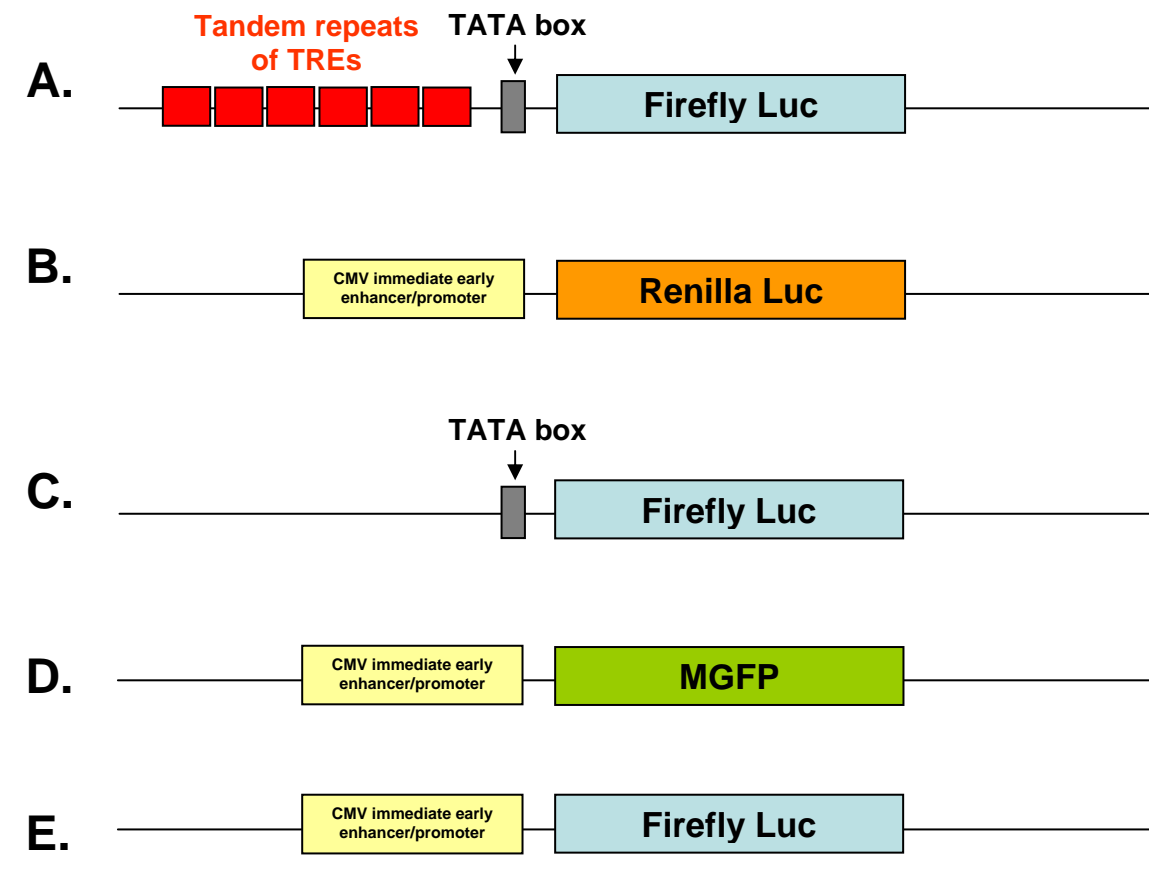


Figure 2: Schematic representation of constructs involved in the Signal Reporter Assay. (A) The inducible transcription factor-responsive construct expressing firefly luciferase, (B) The constitutively expressing *Renilla* luciferase construct, (C) The non-inducible firefly luciferase reporter construct, (D) The constitutively expressing GFP construct, and (E) The constitutively expressing firefly luciferase construct.

B. GFP Reporter Assay Kits:

1. Kit Contents

Table 2: Signal GFP Reporter Assay Kit Specifications

Component	Specification	Concentration and Volume
Reporter	An inducible transcription factor responsive GFP reporter	(100 ng/μl; 500 μl)
Negative control	A GFP reporter construct in which GFP expression is controlled by a minimal promoter	(100 ng/μl; 500 μl)
Positive control	A constitutively expressing GFP construct	(100 ng/μl; 250 μl)

NOTE: These constructs are **transfection-grade** and are ready for transient transfection. These constructs are specifically designed to inhibit transformation and are NOT MEANT for introduction and amplification in bacteria.

2. Description:

Each Signal GFP Reporter Assay Kit includes the following components:

1. **Reporter:** The inducible transcription factor-responsive GFP reporter encodes the green fluorescent protein gene under the control of a basal promoter element (TATA box) joined to tandem repeats of a specific Transcriptional Response Element (TRE; Figure 3A). This construct monitors both increases and decreases in the activity of a key transcription factor, which is a downstream target of a specific signaling pathway
2. **Negative control:** The negative control is a GFP reporter that encodes the green fluorescent protein under the control of a basal promoter element (TATA box), without any additional transcriptional response elements (Figure 3B). The negative control is critical to identifying pathway-specific effects and determining background reporter activity.
3. **Positive control:** The positive control is a constitutively expressing GFP construct (Figure 3C). The positive control is necessary for visual confirmation of transfection. It is also useful for transfection optimization studies.

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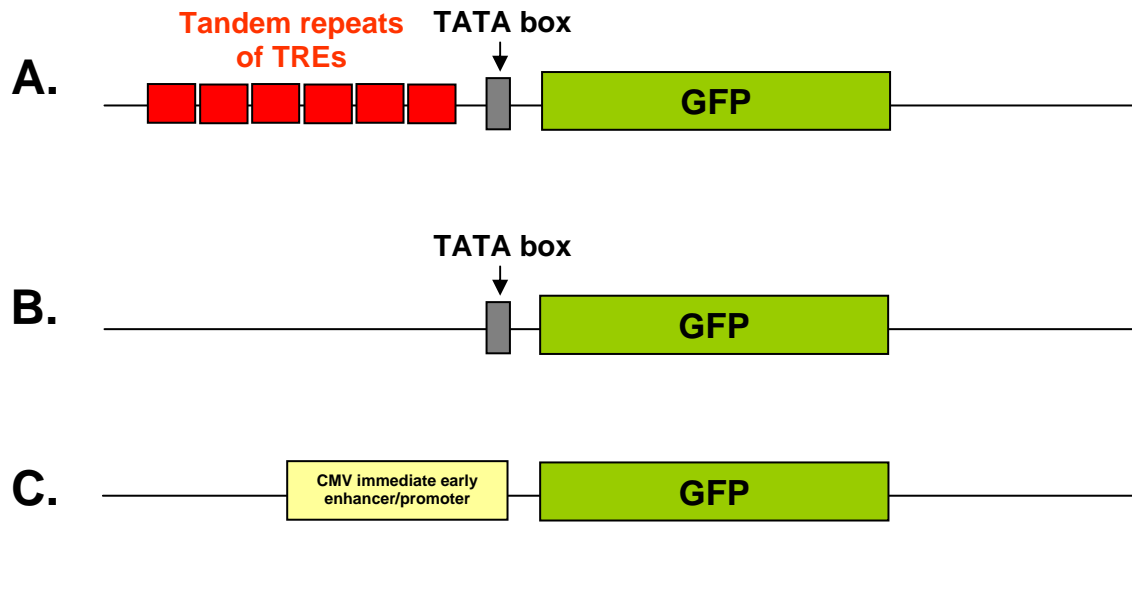


Figure 3: Schematic representation of constructs involved in Cignal GFP Reporter Assay. (A) The inducible transcription factor-responsive reporter expressing GFP, (B) The GFP reporter controlled by a minimal promoter (negative control), (C) The constitutively expressing GFP construct (positive control).

IMPORTANT NOTE: There are a few reports in the literature of the CMV regulatory element being activated by certain stimuli (see below). We recommend that you confirm that the stimulus used in each Cignal reporter assay does not induce the CMV regulatory element, in order to confirm that the CMV-Renilla construct is the appropriate normalization construct for your experiment. This can be done empirically by testing the impact of your stimulus on the Cignal positive control reporters, which are each under the control of the CMV enhancer/promoter cassette. If your stimulus is one of the very few reported activators of the CMV regulatory element, we advise using an alternative reporter as an internal control.

- W. Bruening, B. Giasson, W. Mushynski, and H. D. Durham. 1998. *Nucleic Acids Research* 26(2):486-489. **Activation of stress-activated MAP protein kinases up-regulates expression of transgenes driven by the cytomegalovirus immediate/early promoter**
- Madhu S. Malo, Moushumi Mozumder, Alexander Chen, Golam Mostafa, Xiao Bo Zhang, Richard A. Hodin. 2006. *Analytical Biochemistry* 350:307-309. **pFRL7: An ideal vector for eukaryotic promoter analysis**

III. Additional Materials Required:

- Mammalian cell line cultured in the appropriate growth medium
- Cell culture medium and standard cell culture supplies
- 96-well tissue culture plates
- Multi-channel pipettor and pipettor reservoirs
- Transfection reagent
We recommend SureFECT (SABiosciences) and Attractene (QIAGEN), however, other transfection reagents work equally well
- Polystyrene test tubes (BD FALCON, Cat # 352099)
- Opti-MEM® I Reduced Serum Medium (Invitrogen, Cat. No. 31985-062)
- Fetal bovine serum (FBS)
- Non-essential amino acids (NEAA) (Invitrogen, Cat. No. 11140-050)
- Penicillin/Streptomycin
- Hemacytometer
- Dual-Luciferase® Assay System
 - Dual-Luciferase® Reporter Assay System (Promega, Cat. No. E1910)
This system requires cell lysis, and is well-suited for the rapid quantitation of both luciferase reporters when using luminometers with reagent auto-injectors.
 - Dual-Glo® Luciferase Assay System (Promega, Cat. No. E2920)
This system is used to assay for both luciferase reporters on intact cells in growth medium. This system can be used with any luminometer, including those without reagent auto-injectors.
- 96-well white opaque flat bottom microtiter plate
- Luminometer
- FACS, flow cytometer, fluorescent microscope, or fluorometer

IV. Protocol:

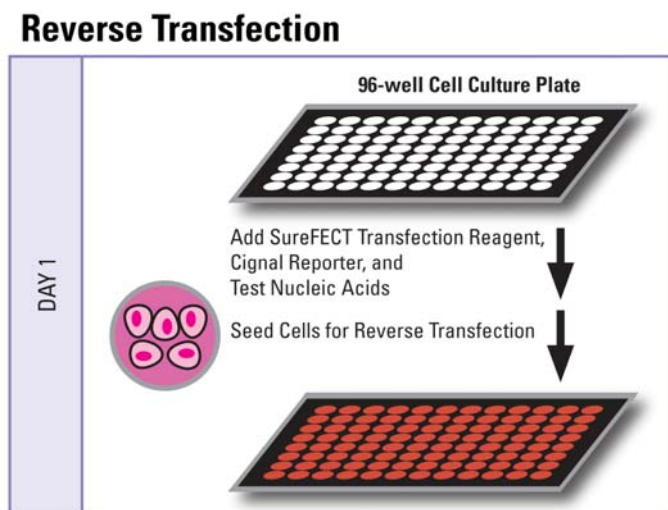
A. Before you begin:

1. Cell line selection: The Signal Reporter Assay may be used with various mammalian cell lines. Cell lines show a great deal of variation in the levels of signaling proteins. The transcriptional activator activities in the cell line used will determine the sensitivity of the assay. A cell line should be selected based on the functionality of the signal transduction pathway under investigation, as well as for the “transfectability” of the cell line (see below).
2. Transfection reagent selection: We recommend the use of SureFECT (SABiosciences) or Attractene (QIAGEN) as a transfection reagent. The Signal Reporter Assay, however, also performs equally well with other transfection reagents such as Lipofectamine 2000 (Invitrogen), or FuGENE 6 (Roche). When using alternative transfection reagents, please refer to the manufacturer’s instructions on the use of those reagents.
3. Optimization of transfection conditions: The sensitivity of the Signal Reporter Assay depends on the transfection efficiency. The transfection efficiency, in turn, primarily depends upon cell line used. Therefore, it is very important to optimize the transfection conditions for each cell type under study. Variables to consider, when optimizing the transfection conditions include cell density, cell viability, amount of DNA, ratio of DNA to transfection reagent, transfection complex formation time, and transfection incubation time (see the detailed protocols for recommendations). The positive control construct included with each Signal Reporter Assay can be used for determining the optimal transfection conditions.
4. Optimization of assay condition: The response rate in the Signal Reporter Assay depends on the assay conditions (conditions of the experimental treatment). To obtain maximum response given by any stimulus, perform dosing and time-course studies. The optimal amount of stimulus and the time of treatment must be obtained empirically for each experiment (see different protocols for recommendations).
5. Important recommendations for best results:
 - A. Perform all transfections in **triplicate** to minimize variability among treatment groups.
 - B. Include positive and negative controls in each experiment to obtain reliable results.
 - C. Use low-passage cells that are actively growing and are greater than 90% viable, for maximal transfection efficiencies.
 - D. Do not add antibiotics to media during transfection, as this may cause cell death.
 - E. Take care to always seed the same number of cells in each well, in order to maximize the reproducibility.
 - F. Serum induces various signaling pathways, leading to cross-talk and high background. Therefore, use reduced amounts of serum (0.5%) in the assay medium during the experimental treatment to minimize these serum effects.

B. Generalized Transfection Protocols:

We recommend using reverse transfection protocols with the SureFECT transfection reagent throughout the Signal Reporter Assays User Manual. This is due to the time savings and improved reproducibility of using this method, compared to traditional forward transfection methods. However, Signal Reporter Assays also work well with traditional forward transfection methods and transfection reagents from other vendors. Below are general protocol overviews for the Signal Reporter Assays, using either reverse or forward transfection approaches.

1. Reverse Transfection Protocol Overview (1 DAY PROCEDURE)



DAY 1

- Prepare nucleic acid mixtures in appropriate ratios. This may include any of the following combinations, depending upon the experimental design (we recommend carrying out each transfection condition in triplicate):

Experimental transfection

- i. Signal Reporter + test nucleic acid (expression plasmids, shRNA plasmids, or siRNAs)

Control transfections

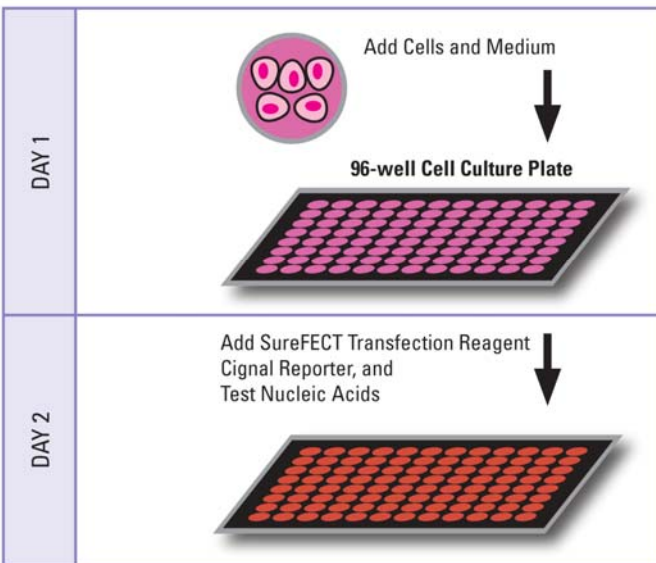
- ii. Signal Reporter + negative control for test nucleic acid
 - iii. Signal Negative Control + test nucleic acid (expression plasmids, shRNA plasmids, or siRNAs)
 - iv. Signal Negative Control + negative control for test nucleic acid
 - v. Signal Positive Control
- Dilute SureFECT into Opti-MEM
 - Add diluted SureFECT to nucleic acid mixtures, incubate at room temperature for 20 minutes
 - Trypsinize (if necessary), count, and suspend cells to appropriate density
 - Aliquot transfection complexes into wells
 - Immediately seed cells to each well *

* For detailed information on the transfection conditions, and treatment of cultures post-transfection, refer to the application-specific protocols within this user manual.

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2. Traditional Transfection Protocol Overview (2 DAY PROCEDURE)

Traditional Transfection



DAY 1

- Trypsinize (if necessary), count, and suspend cells to appropriate density
- Seed cells into multiwell plate(s)

DAY 2

- Prepare nucleic acid mixtures in appropriate ratios. This may include any of the following combinations, depending upon the experimental design (we recommend carrying out each transfection condition in triplicate):

Experimental transfection

- i. Signal Reporter + test nucleic acid (expression plasmids, shRNA plasmids, or siRNAs)

Control transfections

- ii. Signal Reporter + negative control for test nucleic acid
 - iii. Signal Negative Control + test nucleic acid (expression plasmids, shRNA plasmids, or siRNAs)
 - iv. Signal Negative Control + negative control for test nucleic acid
 - v. Signal Positive Control
- Dilute transfection reagent into appropriate medium (**If you are using a transfection reagent other than SureFECT follow their manufacturer's protocol for transfection**)
 - Add diluted transfection reagent to nucleic acid mixtures, incubate at room temperature for 20 minutes
 - Aliquot transfection complexes into wells containing overnight cell cultures

C. Co-transfection Protocol for siRNA + Reporter Assay

The following protocol is designed to reverse transfect adherent cell line, HEK-293H, using SureFECT Transfection Reagent (SABiosciences) in a 96-well plate format. The Cignal Reporter Assay works well with transfection reagents from other vendors. *If you are using a transfection reagent other than SureFECT follow their manufacturer's protocol for optimizing transfection.* The Cignal Reporter Assay also works well using traditional forward transfection protocols. Moreover, if you are using plates or wells of different size, adjust the components in proportion to the surface area (see section IV.H). **This is just a general guideline; the optimal conditions/amounts should be optimized according to the cell type and the study requirements.** Read the protocol completely before starting the experiment.

IMPORTANT: (1) Do not add antibiotics to media during transfection as this causes cell death. (2) Avoid the use of DMEM medium*.

Table 2: Guidelines for setting up co-transfections of siRNA and Cignal Reporter Assays. Table 2 represents the total components needed, on a per well basis, for each condition to be tested. Note that individual components must be added sequentially, as instructed in the protocol.

#	Cignal Reporter (per well)	Cignal Negative Control (per well)	Cignal Positive Control (per well)	Specific siRNA (per well)	Negative Control siRNA (per well)	Opti-MEM Nucleic Acid Diluent (per well)	SureFECT (per well)	Opti-MEM SureFECT Diluent (per well)	Time of transfection (hours)
1	100 ng (1.0 µl)				2 pmol	25 µl	0.3 µl	25 µl	48 h or 72 h
2	100 ng (1.0 µl)			2 pmol		25 µl	0.3 µl	25 µl	
3		100 ng (1.0 µl)			2 pmol	25 µl	0.3 µl	25 µl	
4		100 ng (1.0 µl)		2 pmol		25 µl	0.3 µl	25 µl	
5			100 ng (1.0 µl)			25 µl	0.3 µl	25 µl	

1. The recommended experimental setup, on a **per well basis**, follows. Please note that we recommend setting up multiple replicates for each condition, and preparing transfection cocktail volumes sufficient for transfecting multiple wells. In addition, we advise always taking 5-10% extra amounts of nucleic acid, Opti-MEM® serum-free culture medium, and SureFECT to compensate for pipettor error when setting up transfection cocktails (steps 1 through 4).

Add 25 µl of Opti-MEM® to each of 5 polystyrene tubes (avoid using DMEM*), along with the following:

Experimental transfection

- 1 µl (100 ng) Cignal reporter + 2 pmol sequence-specific siRNA

Control transfections

- 1 µl (100 ng) Cignal reporter + 2 pmol negative control siRNA
- 1 µl (100 ng) Cignal negative control + 2 pmol sequence-specific siRNA
- 1 µl (100 ng) Cignal negative control + 2 pmol negative control siRNA
- 1 µl (100 ng) Cignal positive control

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Mix each transfection cocktail gently.

2. Prepare a SureFECT dilution for 5 tubes (mentioned in step 1) by dispensing 1.5 μ l of SureFECT into 125 μ l of Opti-MEM® serum-free culture medium (for every well dilute 0.3 μ l of SureFECT in 25 μ l of Opti-MEM® serum-free culture medium) in a polystyrene test tube. Mix gently and set the tube at room temperature for 5 minutes.

3. After the 5 minute incubation, add 25 μ l of diluted SureFECT into each of the five tubes containing 25 μ l of the diluted nucleic acids (1:1 ratio) as detailed in Table 2.

4. Mix gently and incubate for 20 minutes at room temperature to allow complex formation to occur.

5. Meanwhile, wash cells** in a culture dish once with Dulbecco's PBS **without calcium and magnesium**, and treat with 1-3 ml trypsin-EDTA for 2-5 minutes at 37°C in a humidified atmosphere containing 5% CO₂. Suspend the cells in 7-9 ml of Opti-MEM® containing 5% of fetal bovine serum, then centrifuge the cells down, remove the supernatant, and resuspend the cells to 2×10^5 cells/ml in Opti-MEM® containing 5% of fetal bovine serum and 1% NEAA***. To ensure reproducible transfection results, it is important to accurately measure the cell density with a hemacytometer or an automated cytometry device.

6. After the 20 minute incubation for complex formation is completed, aliquot 50 μ l of specific constructs-siRNA-SureFECT complexes into the appropriate wells.

7. Add 100 μ l of prepared cell suspension (2×10^5 cells/ml in Opti-MEM® containing 5% of fetal bovine serum) to each well containing constructs-siRNA-SureFECT complexes. This gives a final volume of 150 μ l. Mix gently by rocking the plate back and forth.

8. Incubate cells at 37°C in a 5% CO₂ incubator for 16-24 hours.

9. After 16-24 hours of transfection, change the medium to complete growth medium (DMEM with 10% FBS, 0.1mM NEAA, 1mM Sodium pyruvate, 100 U/ml penicillin and 100 μ g/ml streptomycin).

10. To study the effect of knockdown, we recommend harvesting cells 48 or 72 hours after transfection to perform dual-luciferase assay.

12. The **luciferase assay** can be developed by using Dual-Luciferase Reporter Assay System from Promega (Cat. No. 1910). Follow the manufacturer's protocol for developing the assay.

Expression of the **Monster GFP** reporter can be monitored via FACS, flow cytometry, fluorescent microscopy, or standard fluorometry. The spectral properties of the Monster Green Fluorescent Protein are slightly red-shifted compared to other commercially available GFP reporters. We recommend using the standard FACS settings of an argon laser (488nm excitation) and filters of 530 ± 15 nm (530/30nm) for emission. When analyzing GFP expression via fluorescent microscopy or standard fluorometry, we recommend using standard fluoroisothiocyanate (FITC) filters [excitation of 470 ± 20 nm and an emission filter of 515nm (long pass)].

* We have found that some components in DMEM interfere with SureFECT transfection protocol. However, DMEM has no effect on the performance of Signal Reporter Assays.

**Cells that have been passed 1:3 or 1:4 the day before are generally more easily transfected than cells that have reached a confluent state at the time of use.

***In most cases, cells grow well in Opti-MEM® serum-reduced growth medium with 3-5% FBS due to extra growth factors and nutrients supplied in Opti-MEM®. Cell should reach ~50-90% confluence once attached to the wells, otherwise increase the cell numbers.

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D. Co-transfection Protocol for shRNA + Reporter Assay

The following protocol is designed to reverse transfect adherent cell line, HEK-293H, using SureFECT Transfection Reagent (SABiosciences) in a 96-well plate format. The Signal Reporter Assay works well with transfection reagent from other vendors. *If you are using transfection reagent other than SureFECT follow their manufacturer's protocol for transfection.* The Signal Reporter Assay also works well using traditional forward transfection protocols. Moreover, if you are using plates or wells of different size, adjust the components in proportion to the surface area (see section IV.H). **This is just a general guideline; the optimal conditions/amounts should be adjusted according to the cell type and study requirements.** Read the protocol completely before starting the experiment.

IMPORTANT: (1) Do not add antibiotics to media during transfection as this causes cell death. (2) Avoid the use of DMEM medium*.

Table 3: Guidelines for setting up co-transfections of a shRNA vector and Signal Reporter Assay. Table 3 represents the total components needed, on a per well basis, for each condition to be tested. Note that individual components must be added sequentially, as instructed in the protocol.

#	Signal Reporter (per well)	Signal Negative Control (per well)	Signal Positive Control (per well)	Specific shRNA (per well)	Negative Control shRNA (per well)	Opti-MEM Nucleic Acid Diluent (per well)	SureFECT (per well)	Opti-MEM SureFECT Diluent (per well)	Time of transfection (hours)
1	100 ng (1.0 μ l)				200 ng	25 μ l	0.3 μ l	25 μ l	48 h or 72 h
2	100 ng (1.0 μ l)			200 ng		25 μ l	0.3 μ l	25 μ l	
3		100 ng (1.0 μ l)			200 ng	25 μ l	0.3 μ l	25 μ l	
4		100 ng (1.0 μ l)		200 ng		25 μ l	0.3 μ l	25 μ l	
5			100 ng (1.0 μ l)			25 μ l	0.3 μ l	25 μ l	

1. The recommended experimental setup, on a **per well basis**, follows. Please note that we recommend setting up multiple replicates for each condition, and preparing transfection cocktail volumes sufficient for transfecting multiple wells. In addition, we advise always taking 5-10% extra amounts of nucleic acid, Opti-MEM® serum-free culture medium, and SureFECT to compensate for pipettor error when setting up transfection cocktails (steps 1 through 4).

Add 25 μ l of Opti-MEM® to each of 5 polystyrene tubes (avoid using DMEM*), along with the following:

Experimental transfection

- 1 μ l (100 ng) Signal reporter + 200 ng sequence-specific shRNA

Control transfections

- 1 μ l (100 ng) Signal reporter + 200 ng negative control shRNA
- 1 μ l (100 ng) Signal negative control + 200 ng sequence-specific shRNA
- 1 μ l (100 ng) Signal negative control + 200 ng negative control shRNA
- 1 μ l (100 ng) Signal positive control

Mix each transfection cocktail gently.

2. Prepare a SureFECT dilution for 5 tubes (mentioned in step 1) by dispensing 1.5 μ l of SureFECT into 125 μ l of Opti-MEM® serum-free culture medium (for every well dilute 0.3 μ l of SureFECT in 25 μ l of Opti-MEM® serum-free culture medium) in a polystyrene test tube. Mix gently and set the tube at room temperature for 5 minutes.

3. After the 5 minute incubation, add 25 μ l of diluted SureFECT into each of the five tubes containing 25 μ l of diluted constructs (1:1 ratio) as detailed in Table 3.

4. Mix gently and incubate for 20 minutes at room temperature to allow complex formation to occur.

5. Meanwhile, wash cells** in a culture dish once with Dulbecco's PBS **without calcium and magnesium**, and treat with 1-3 ml trypsin-EDTA for 2-5 minutes at 37°C in a humidified atmosphere containing 5% CO₂. Suspend the cells in 7-9 ml of Opti-MEM® containing 5% of fetal bovine serum, then centrifuge the cells down, remove the supernatant, and resuspend the cells to 2×10^5 cells/ml in Opti-MEM® containing 5% of fetal bovine serum and 1% NEAA***. To ensure reproducible transfection results, it is important to accurately measure the cell density with a hemacytometer or automated cytometry device.

6. After the 20 minute incubation for complex formation is completed, aliquot 50 μ l of specific constructs-shRNA-SureFECT complexes into the appropriate wells.

7. Add 100 μ l of prepared cell suspension (2×10^5 cells/ml in Opti-MEM® containing 5% of fetal bovine serum) to each well containing construct-shRNA-SureFECT complexes. This gives a final volume of 150 μ l. Mix gently by rocking the plate back and forth.

8. Incubate cells at 37°C in a 5% CO₂ incubator for 16-24 hours.

9. After 16-24 hours of transfection, change the medium to complete growth medium (DMEM with 10% FBS, 0.1mM NEAA, 1mM Sodium pyruvate, 100 U/ml penicillin and 100 μ g/ml streptomycin).

10. To study the effect of knockdown, we recommend harvesting cells 48 or 72 hours after transfection to perform dual-luciferase assay.

11. The **luciferase assay** can be developed by using Dual-Luciferase Reporter Assay System from Promega (Cat. No. 1910). Follow the manufacturer's protocol for developing the assay.

Expression of the **Monster GFP** reporter can be monitored via FACS, flow cytometry, fluorescent microscopy, or standard fluorometry. The spectral properties of the Monster Green Fluorescent Protein are slightly red-shifted compared to other commercially available GFP reporters. We recommend using the standard FACS settings of an argon laser (488nm excitation) and filters of 530 ± 15 nm (530/30nm) for emission. When analyzing GFP expression via fluorescent microscopy or standard fluorometry, we recommend using standard fluorescein isothiocyanate (FITC) filters [excitation of 470 ± 20 nm and an emission filter of 515nm (long pass)].

* We have found that some components in DMEM interfere with SureFECT transfection protocol. However, DMEM has no effect on the performance of Signal Reporter Assays.

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**Cells that have been passed 1:3 or 1:4 the day before are generally more easily transfected than cells that have reached a confluent state at the time of use.

***In most cases, cells grow well in Opti-MEM® serum reduced growth medium with 3%-5% FBS due to extra growth factors and nutrients supplied in Opti-MEM®. Cell should reach ~50-90% confluence once attached to the wells, otherwise increase the cell numbers.

E. Co-transfection Protocol for Expression Vector + Reporter Assay

The following protocol is designed to reverse transfect adherent cell line, HEK-293H, using SureFECT Transfection Reagent (SABiosciences) in a 96-well plate format. The Cignal Reporter Assay works well with transfection reagent from other vendors. *If you are using transfection reagent other than SureFECT follow their manufacturer's protocol for transfection.* The Cignal Reporter Assay also works well using traditional forward transfection protocols. Moreover, if you are using plates or wells of different size, adjust the component in proportion to the surface area (see section IV.H). **This is just a general guideline; the optimal conditions/amounts should be adjusted according to the cell type and the study requirements.** Read the protocol completely before starting the experiment.

IMPORTANT: (1) Do not add antibiotics to media during transfection as this causes cell death. (2) Avoid the use of DMEM medium*.

Table 4: Guidelines for setting up co-transfections of an expression vector and Cignal Reporter Assay. Table 4 represents the total components needed, on a per well basis, for each condition to be tested. Note that individual components must be added sequentially, as instructed in the protocol.

#	Cignal Reporter (per well)	Cignal Negative Control (per well)	Cignal Positive Control (per well)	Experimental Vector with Gene of Interest (per well)	Experimental Vector Without Insert (per well)	Carrier DNA ^a	Opti-MEM Nucleic Acid Diluent (per well)	SureFECT (per well)	Opti-MEM SureFECT Diluent (per well)	Time of transfection (hours)
1	100 ng (1.0 µl)				100 ng	100 ng	25 µl	0.3 µl	25 µl	32 h - 48 h
2	100 ng (1.0 µl)				200 ng		25 µl	0.3 µl	25 µl	
3	100 ng (1.0 µl)			100 ng		100 ng	25 µl	0.3 µl	25 µl	
4	100 ng (1.0 µl)			200 ng			25 µl	0.3 µl	25 µl	
5		100 ng (1.0 µl)			100 ng	100 ng	25 µl	0.3 µl	25 µl	
6		100 ng (1.0 µl)			200 ng		25 µl	0.3 µl	25 µl	
7		100 ng (1.0 µl)		100 ng		100 ng	25 µl	0.3 µl	25 µl	
8		100 ng (1.0 µl)		200 ng			25 µl	0.3 µl	25 µl	
9			100 ng (1.0 µl)				25 µl	0.3 µl	25 µl	

^aCarrier DNA means any empty plasmid, such as a pUC or a pBR plasmid.

1. The recommended experimental setup, on a **per well basis**, follows. Please note that we recommend setting up multiple replicates for each condition, and preparing transfection cocktail volumes sufficient for transfecting multiple wells. In addition, we advise always taking 5-10% extra amounts of nucleic acid, Opti-MEM® serum-free culture medium, and SureFECT to compensate for pipettor error when setting up transfection cocktails (steps 1 through 4).

Signal Reporter Assays

Add 25 μ l of Opti-MEM® to each of 9 polystyrene tubes (avoid using DMEM*), along with the following:

Experimental transfections

- 1 μ l (100 ng) Signal reporter + 100 ng experimental vector expressing gene of interest + 100 ng carrier DNA
- 1 μ l (100 ng) Signal reporter + 200 ng experimental vector expressing gene of interest

Control transfections

- 1 μ l (100 ng) Signal reporter + 100 ng negative control expression vector + 100 ng carrier DNA
- 1 μ l (100 ng) Signal reporter + 200 ng negative control expression vector
- 1 μ l (100 ng) Signal negative control + 100 ng experimental vector expressing gene of interest + 100 ng carrier DNA
- 1 μ l (100 ng) Signal negative control + 200 ng experimental vector expressing gene of interest
- 1 μ l (100 ng) Signal negative control + 100 ng negative control expression vector + 100 ng carrier DNA
- 1 μ l (100 ng) Signal negative control + 200 ng negative control expression vector
- 1 μ l (100 ng) Signal positive control

Mix each transfection cocktail gently.

2. Prepare a SureFECT dilution for 9 tubes (mentioned in step 1) by dispensing 2.7 μ l of SureFECT into 225 μ l of Opti-MEM® serum-free culture medium (for every well dilute 0.3 μ l of SureFECT in 25 μ l of Opti-MEM® serum-free culture medium) in a polystyrene test tube. Mix gently and set the tube at room temperature for 5 minutes.

3. After the 5 minute incubation, add 25 μ l of diluted SureFECT into each of the nine tubes containing 25 μ l of diluted constructs (1:1 ratio) as detailed in Table 4.

4. Mix gently and incubate for 20 minutes at room temperature to allow complex formation to occur.

5. Meanwhile, wash cells** in a culture dish once with Dulbecco's PBS **without calcium and magnesium**, and treat with 1-3 ml trypsin-EDTA for 2-5 minutes at 37°C in a humidified atmosphere containing 5% CO₂. Suspend the cells in 7-9 ml of Opti-MEM® containing 5% of fetal bovine serum, then centrifuge the cells down, remove the supernatant, and resuspend the cells to 2×10^5 cells/ml in Opti-MEM® containing 5% of fetal bovine serum and 1% NEAA***. To ensure reproducible transfection results, it is important to accurately measure the cell density with a hemacytometer or automated cytometry device.

6. After the 20 minute incubation for complex formation is completed, aliquot 50 μ l of specific complexes into the appropriate wells.

7. Add 100 μ l of prepared cell suspension (2×10^5 cells/ml in Opti-MEM® containing 5% of fetal bovine serum) to each well containing construct-vector-SureFECT complexes. This gives a final volume of 150 μ l. Mix gently by rocking the plate back and forth.

8. Incubate cells at 37°C in a 5% CO₂ incubator for 16-24 hours.

9. After 16-24 hours of transfection, change the medium to complete growth medium (DMEM with 10% FBS, 0.1mM NEAA, 1mM Sodium pyruvate, 100 U/ml penicillin and 100 µg/ml streptomycin).

11. To study the effect of the gene product, we recommend harvesting cells 32 hours or 48 hours after transfection to perform the dual-luciferase assay.

12. The **luciferase assay** can be developed by using Dual-Luciferase Reporter Assay System from Promega (Cat. No. 1910). Follow the manufacturer's protocol for developing the assay.

Expression of the **Monster GFP** reporter can be monitored via FACS, flow cytometry, fluorescent microscopy, or standard fluorometry. The spectral properties of the Monster Green Fluorescent Protein are slightly red-shifted compared to other commercially available GFP reporters. We recommend using the standard FACS settings of an argon laser (488nm excitation) and filters of 530±15 nm (530/30nm) for emission. When analyzing GFP expression via fluorescent microscopy or standard fluorometry, we recommend using standard fluorescein isothiocyanate (FITC) filters [excitation of 470±20nm and an emission filter of 515nm (long pass)].

* We have found that some components in DMEM interfere with SureFECT transfection protocol. However, DMEM has no effect on the performance of Signal Reporter Assays.

**Cells that have been passed 1:3 or 1:4 the day before are generally more easily transfected than cells that have reached a confluent state at the time of use.

***In most cases, cells grow well in Opti-MEM® serum reduced growth medium with 3%-5% FBS due to extra growth factors and nutrients supplied in Opti-MEM®. Cell should reach ~50-90% confluence once attached to the wells, otherwise increase the cell numbers.

Signal Reporter Assays

F. Transfection and Treatment Protocol for Reporter Assay + Small Molecules/Organic Compounds

The following protocol is designed to reverse transfect adherent cell line, HEK-293H, using SureFECT (SABiosciences) as a transfection reagent in 96-well plate format. The Signal reporter assay works well with transfection reagent from other vendors. *If you are using transfection reagent other than SureFECT follow their manufacturer's protocol for transfection.* The Signal Reporter Assay also works well using traditional forward transfection protocols. Moreover, if you are using plates or wells of different size, adjust the component in proportion to the surface area (see section IV.H). **This is just a general guideline; the optimal conditions/amounts should be adjusted according to the cell type and the study requirements.** Read the protocol completely before starting the experiment.

IMPORTANT: (1) Do not add antibiotics to media during transfection as this causes cell death. (2) Avoid the use of DMEM* medium*.

Table 5: Guidelines for studying the effect of small molecules/organic compounds.

Table 5 represents the total components needed, on a per well basis, for each condition to be tested. Note that individual components must be added sequentially, as instructed in the protocol.

#	Signal Reporter (per well)	Signal Negative Control (per well)	Signal Positive Control Construct (per well)	Small Molecule/Organic Compound (per well)	Opti-MEM DNA diluent (per well)	SureFECT (per well)	Opti-MEM SureFECT diluent (per well)	Time of Transfection (hours)
1	100 ng (1.0 µl)				25 µl	0.3 µl	25 µl	30 h or 42 h
2	100 ng (1.0 µl)			1X ^a	25 µl	0.3 µl	25 µl	
3	100 ng (1.0 µl)			10X	25 µl	0.3 µl	25 µl	
4	100 ng (1.0 µl)			100X	25 µl	0.3 µl	25 µl	
5		100 ng (1.0 µl)			25 µl	0.3 µl	25 µl	
6		100 ng (1.0 µl)		1X	25 µl	0.3 µl	25 µl	
7		100 ng (1.0 µl)		10X	25 µl	0.3 µl	25 µl	
8		100 ng (1.0 µl)		100X	25 µl	0.3 µl	25 µl	
9			100 ng (1.0 µl)		25 µl	0.3 µl	25 µl	

^a 1X is a smallest appropriate amount of small molecule or organic compound expected to modulate signaling pathway.

1. The recommended experimental setup, on a **per well basis**, follows. Please note that we recommend setting up multiple replicates for each condition, and preparing transfection cocktail volumes sufficient for transfecting multiple wells. In addition, we advise always taking 5-10% extra amounts of nucleic acid, Opti-MEM® serum-free culture medium, and SureFECT to compensate for pipettor error when setting up transfection cocktails (steps 1 through 4).

Set up three polystyrene tubes, as follows

Experimental transfections

Tubes 1 - 4: 100 μ l Opti-MEM® + 4 μ l (400 ng) Cignal reporter (**4 volumes for conditions 1 to 4 of Table 5**; for every well dilute 1 μ l (100 ng) of Cignal reporter in 25 μ l of Opti-MEM® serum-free culture medium)

Control transfections

Tubes 5 - 8: 100 μ l Opti-MEM® + 4 μ l (400 ng) Cignal negative control (**4 volumes for conditions 5 to 8 of Table 5**; for every well dilute 1 μ l (100 ng) of Cignal reporter in 25 μ l of Opti-MEM® serum-free culture medium)

Tube 9: 25 μ l Opti-MEM® + 1 μ l (100 ng) Cignal positive control

Mix each transfection cocktail gently.

2. Prepare a SureFECT dilution for 9 tubes (mentioned in step 1) by dispensing 2.7 μ l of SureFECT into 225 μ l of Opti-MEM® serum-free culture medium (for every well dilute 0.3 μ l of SureFECT in 25 μ l of Opti-MEM® serum-free culture medium) in a polystyrene test tube. Mix gently and set the tube at room temperature for 5 minutes.

3. After the 5 minute incubation, add 100 μ l of diluted SureFECT to the two tubes (mentioned in step 1) containing equal volume (100 μ l) of diluted Cignal reporter, and add 25 μ l of diluted SureFECT into the positive control tube containing 25 μ l of diluted constructs (1:1 ratio) as detailed in Table 5.

4. Mix gently and incubate for 20 minutes at room temperature to allow complex formation to occur.

5. Meanwhile, wash cells** in culture dish once with Dulbecco's PBS **without calcium and magnesium**, and treat with 1-3 ml trypsin-EDTA for 2-5 minutes at 37 °C in a humidified atmosphere containing 5% CO₂. Suspend the cells in 7-9 ml of Opti-MEM® containing 5% of fetal bovine serum, then centrifuge the cells down, remove the supernatant, and resuspend the cells to 2×10^5 cells/ml in Opti-MEM® containing 5% of fetal bovine serum and 1% NEAA***. To ensure reproducible transfection results, it is important to accurately determine the cell density with a hemacytometer or an automated cytometry device.

6. After the 20 minute incubation for complex formation is completed, aliquot 50 μ l of specific complexes into the appropriate wells.

7. Add 100 μ l of prepared cell suspension (2×10^5 cells/ml in Opti-MEM® containing 5% of fetal bovine serum) to each well containing constructs-SureFECT complexes. This gives a final volume of 150 μ l. Mix gently by rocking the plate back and forth.

8. Incubate cells at 37°C in a 5% CO₂ incubator for 16 hours.

9. After 16 hours of transfection, change medium to assay medium (Opti-MEM® containing 0.5% of fetal bovine serum, 1% NEAA, 100 U/ml Penicillin and 100 μ g/ml Streptomycin).

Signal Reporter Assays

10. After 24 hours of transfection, treat the cells, as described in Table 5, with 1×, 10× and 100× amount of small molecule or organic compound (1× is the lowest appropriate amount of small molecule or organic compound expected to modulate the signaling pathway).

11. To study the effect of small molecule or organic compound, we recommend harvesting cells 6 hours or 18 hours after treatment to perform dual-luciferase assay.

12. The **luciferase assay** can be developed by using Dual-Luciferase Reporter Assay System from Promega (Cat. No. 1910). Follow the manufacturer's protocol for developing the assay.

Expression of the **Monster GFP** reporter can be monitored via FACS, flow cytometry, fluorescent microscopy, or standard fluorometry. The spectral properties of the Monster Green Fluorescent Protein are slightly red-shifted compared to other commercially available GFP reporters. We recommend using the standard FACS settings of an argon laser (488nm excitation) and filters of 530±15 nm (530/30nm) for emission. When analyzing GFP expression via fluorescent microscopy or standard fluorometry, we recommend using standard fluorescein isothiocyanate (FITC) filters [excitation of 470±20nm and an emission filter of 515nm (long pass)].

* We found some components in DMEM interfere with the SureFECT transfection protocol. However, DMEM has no effect on the performance of Signal Reporter Assays.

**Cells that had been passed 1:3 or 1:4 the day before are generally more easily transfected than cells that have reached a confluent state at the time of use.

***In most cases, cells grow well in Opti-MEM® serum reduced growth medium with 3%-5% FBS due to extra growth factors and nutrients supplied in Opti-MEM®. Cell should reach ~50-90% confluence once attached to the wells, otherwise increase the cell numbers.

G. Transfection and Treatment Protocol for Reporter Assay + Peptide/Recombinant Protein

The following protocol is designed to reverse transfect adherent cell line, HEK-293H, using SureFECT Transfection Reagent (Cat. No. SA-01) in a 96-well plate format. The Cignal reporter assay works well with transfection reagent from other vendors. *If you are using transfection reagent other than SureFECT follow their manufacturer's protocol for transfection.* The Cignal Reporter Assay also works well using traditional forward transfection protocols. Moreover, if you are using plates or wells of different size, adjust the component in proportion to the surface area (see section IV.F). **This is just a general guideline; the optimal conditions/amounts should be adjusted according to the cell type and the study requirements.** Read the protocol completely before starting the experiment.

IMPORTANT: (1) Do not add antibiotics to media during transfection as this causes cell death. (2) Avoid the use of DMEM* medium*.

Table 6: Guidelines for studying the effect of a peptide or recombinant protein. Table 6 represents the total components needed, on a per well basis, for each condition to be tested. Note that individual components must be added sequentially, as instructed in the protocol.

#	Cignal Reporter (per well)	Cignal Negative Control (per well)	Cignal Positive Control (per well)	Peptide or Recombinant Protein (per well)	Opti-MEM DNA Diluent (per well)	SureFECT (per well)	Opti-MEM SureFECT diluent (per well)	Time of Transfection (hours)
1	100 ng (1.0 µl)				25 µl	0.3 µl	25 µl	30 h or 42 h
2	100 ng (1.0 µl)			1× ^a	25 µl	0.3 µl	25 µl	
3	100 ng (1.0 µl)			10×	25 µl	0.3 µl	25 µl	
4	100 ng (1.0 µl)			100×	25 µl	0.3 µl	25 µl	
5		100 ng (1.0 µl)			25 µl	0.3 µl	25 µl	
6		100 ng (1.0 µl)		1×	25 µl	0.3 µl	25 µl	
7		100 ng (1.0 µl)		10×	25 µl	0.3 µl	25 µl	
8		100 ng (1.0 µl)		100×	25 µl	0.3 µl	25 µl	
9			100 ng (1.0 µl)		25 µl	0.3 µl	25 µl	

^a 1X is a smallest appropriate amount of interfering peptide/recombinant protein/growth factor expected to modulate signaling pathway.

1. The recommended experimental setup, on a **per well basis**, follows. Please note that we recommend setting up multiple replicates for each condition, and preparing transfection cocktail volumes sufficient for transfecting multiple wells. In addition, we advise always taking 5-10% extra amounts of nucleic acid, Opti-MEM® serum-free culture medium, and SureFECT to compensate for pipettor error, when setting up transfection cocktails (steps 1 through 4).

Signal Reporter Assays

Set up three polystyrene tubes, as follows:

Experimental transfections

Tubes 1 – 4: 100 μ l Opti-MEM® + 4 μ l (400 ng) Signal reporter (**4 volumes for conditions 1 to 4 of Table 6**; for every well dilute 1 μ l (100 ng) of Signal reporter in 25 μ l of Opti-MEM® serum-free culture medium)

Control transfections

Tubes 5-8: 100 μ l Opti-MEM® + 4 μ l (400 ng) Signal negative control (**4 volumes for conditions 5 to 8 of Table 6**; for every well dilute 1 μ l (100 ng) of Signal reporter in 25 μ l of Opti-MEM® serum-free culture medium)

Tube 9: 25 μ l Opti-MEM® + 1 μ l (100 ng) Signal positive control

Mix each transfection cocktail gently.

2. Prepare a SureFECT dilution for 9 tubes (mentioned in step 1) by dispensing 2.7 μ l of SureFECT into 225 μ l of Opti-MEM® serum-free culture medium (for every well dilute 0.3 μ l of SureFECT in 25 μ l of Opti-MEM® serum-free culture medium) in a polystyrene test tube. Mix gently and set the tube at room temperature for 5 minutes.

3. After the 5 minute incubation, add 100 μ l of diluted SureFECT to the two tubes (mentioned in step 1) containing equal volume (100 μ l) of diluted Signal reporter, and add 25 μ l of diluted SureFECT into the positive control tube containing 25 μ l of diluted constructs (1:1 ratio) as detailed in Table 6.

4. Mix gently and incubate for 20 minutes at room temperature to allow complex formation to occur.

5. Meanwhile, wash cells** in culture dish once with Dulbecco's PBS **without calcium and magnesium**, and treat with 1-3 ml trypsin-EDTA for 2-5 minutes at 37 °C in a humidified atmosphere containing 5% CO₂. Suspend the cells in 7-9 ml of Opti-MEM® containing 5% of fetal bovine serum, then centrifuge the cells down, remove the supernatant, and resuspend the cells to 2×10^5 cells/ml in Opti-MEM® containing 5% of fetal bovine serum and 1% NEAA***. To ensure reproducible transfection results, it is important to accurately determine the cell density with a hemacytometer or an automated cytometry device.

6. After the 20 minute incubation for complex formation is completed, aliquot 50 μ l of specific complexes into the appropriate wells.

7. Add 100 μ l of prepared cell suspension (2×10^5 cells/ml in Opti-MEM® containing 5% of fetal bovine serum) to each well containing constructs-SureFECT complexes. This gives a final volume of 150 μ l. Mix gently by rocking the plate back and forth.

8. Incubate cells at 37°C in a 5% CO₂ incubator for 16 hours.

9. After 16 hours of transfection, change medium to assay medium (Opti-MEM® containing 0.5% of fetal bovine serum, 1% NEAA, 100 U/ml Penicillin and 100 μ g/ml Streptomycin).

10. After 24 hours of transfection, treat the cells, as described in Table 12, with 1×, 10× and 100× amount interfering peptide/recombinant protein/growth factor (1× is an smallest appropriate amount of small molecule or organic compound expected to modulate signaling pathway).

11. To study the effect of interfering peptide/recombinant protein/growth factor, we recommend harvesting cells 6 hours or 18 hours after treatment to develop luciferase assay.

12. The **luciferase assay** can be developed by using Dual-Luciferase Reporter Assay System from Promega (Cat. No. 1910). Follow the manufacturer's protocol for developing the assay.

Expression of the **Monster GFP** reporter can be monitored via FACS, flow cytometry, fluorescent microscopy, or standard fluorometry. The spectral properties of the Monster Green Fluorescent Protein are slightly red-shifted compared to other commercially available GFP reporters. We recommend using the standard FACS settings of an argon laser (488nm excitation) and filters of 530±15 nm (530/30nm) for emission. When analyzing GFP expression via fluorescent microscopy or standard fluorometry, we recommend using standard fluorescein isothiocyanate (FITC) filters [excitation of 470±20nm and an emission filter of 515nm (long pass)].

* We found some components in DMEM interfere with SureFECT transfection protocol. However, DMEM has no effect on the performance of Signal Reporter Assays.

**Cells that had been passed 1:3 or 1:4 the day before are generally more easily transfected than cells that have reached a confluent state at the time of use.

***In most cases, cells grow well in Opti-MEM® serum reduced growth medium with 3%-5% FBS due to extra growth factors and nutrients supplied in Opti-MEM®. Cell should reach ~50-90% confluence once attached to the wells, otherwise increase the cell numbers.

Signal Reporter Assays

H. Scaling up transfection experiments:

To transfect cells in different tissue culture formats, vary the amounts of constructs, number of cells, and volume of SureFECT and medium used in proportion to the surface area, as shown in the Table 7. The parameters shown in Table 7 are standardized for HEK-293H cells. **Use these parameters as a starting point to optimize transfections for your cell line of interest.**

Table 7. Reagent amounts for transfecting cells in different size culture vessels

Type of Plate	Surface Area (cm ² per well)	Starting amount of construct (ng / well)	Starting Volume of SureFECT (μl / well)	Volume of Cell Suspension (μl / well)	Starting No. of Adherent Cells (per Well)	Volume of Opti-MEM Medium (μl)	siRNA / shRNA Vector or Gene Expression Vector (per Well)
96-well	0.3	100	0.3	100	20,000	2 X 25 ^a	2 pmol / 200 ng
48-well	0.95	150	0.8	250	62,500	2 X 50	5 pmol / 500 ng
24-well	1.9	250	1.6	500	125,000	2 X 50	10 pmol / 750 ng
12-well	3.8	500	3.2	1000	250,000	2 X 100	20 pmol / 1.5 μg
6-well	9.4	1000	8.0	2500	750,000	2 X 250	50 pmol / 4.0 μg
35 mm	8.0	1000	8.0	2500	750,000	2 X 250	50 pmol / 4.0 μg
60mm	21	2000	18.0	5000	1.5X 10 ⁶	2 X 500	100 pmol / 8.0 μg
100mm	55	5000	45.0	15000 (15ml)	4.5X 10 ⁶	2 X 1500	300 pmol / 25 μg

^a 2X means one volume of Opti-MEM® medium for diluting constructs and another volume of Opti-MEM® medium for diluting SureFECT.

For any other troubleshooting or technical questions about the Signal Reporter Assay, please call one of our Technical Support representatives at 1-888-503-3187 or 301-682-9200 or email at support@SABiosciences.com.

Appendix:

Signal Reporter Assay Kits

Pathway	Transcription Factor	Dual luciferase Cat. No.	MGFP Cat. No.
Amino Acid Deprivation	ATF4/ATF3/ATF2	CCS-8034L	
Androgen	Androgen Receptor	CCS-1019L	
Antioxidant Response	Nrf2 & Nrf1	CCS-5020L	CCS-0020G
ATF6	ATF6	CCS-9031L	
C/EBP	C/EBP	CCS-001L	CCS-1001G
cAMP/PKA	CREB	CCS-002L	CCS-002G
Cell Cycle	E2F/DP1	CCS-003L	
DNA Damage	p53	CCS-004L	
Early Growth Response	EGR1	CCS-8021L	CCS-3021G
ER Stress	CBF/NF- γ /YY1	CCS-2032L	
Estrogen Receptor	Estrogen Receptor (ER)	CCS-005L	
GATA	GATA	CCS-1035L	
Glucocorticoid Receptor	Glucocorticoid Receptor (GR)	CCS-006L	
Heat Shock Response	HSF	CCS-4023L	
Heavy Metal Response	MTF1	CCS-5033L	CCS-0033G
Hedgehog	Gli	CCS-6030L	
Hepatocyte Nuclear Factor 4	HNF4	CCS-3039L	
Hypoxia	HIF-1	CCS-007L	
Interferon Regulation	IRF-1	CCS-7040L	
Interferon Type I	STAT1/STAT2	CCS-008L	CCS-008G
Interferon Gamma	STAT1/STAT1	CCS-009L	
KLF4	KLF4	CCS-4036L	CCS-9036G
Liver X Receptor	LXR α	CCS-0041L	
MAPK/ERK	Elk-1/SRF	CCS-010L	CCS-010G
MAPK/JNK	AP-1	CCS-011L	CCS-011G
MEF2	MEF2	CCS-7024L	
c-Myc	Myc/Max	CCS-012L	
Nanog	Nanog	CCS-7037L	
NF κ B	NF κ B	CCS-013L	CCS-013G
Notch	RBP-J κ	CCS-014L	CCS-1014G
Oct4	Oct4	CCS-0025L	
Pax6	Pax6	CCS-3042L	
PI3K/AKT	FOXO	CCS-1022L	CCS-6022G
PKC/Ca ⁺⁺	NFAT	CCS-015L	
PPAR	PPAR	CCS-3026L	
Progesterone	Progesterone Receptor	CCS-6043L	
Retinoic Acid Receptor	RAR	CCS-016L	
Retinoid X Receptor	RXR	CCS-9044L	
Sox2	Sox2	CCS-0038L	
SP1	SP1	CCS-6027L	CCS-1027G
STAT3	STAT3	CCS-9028	
TGF β	SMAD2/SMAD3/SMAD4	CCS-017L	CCS-017G
Vitamin D	VDR	CCS-2029L	
Wnt	TCF/LEF	CCS-018L	CCS-018G
Xenobiotic	AhR	CCS-2045L	CCS-7045G

Signal Reporter Assays

Signal Reporter Assays

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replace this product in the event the product fails to perform due to any manufacturing defect. SABiosciences Corporation makes no other warranties of any kind, expressed or implied, including without limitation, warranties of merchantability or fitness for a particular purpose. SABiosciences Corporation shall not be liable for any direct, indirect, consequential or incidental damages arising out of the use, the results of use or the inability to use this product.
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For research use only. The terms of the limited license conveyed with the purchase of this product are as follows: Researchers may use this product in their own research and they may transfer derivatives to others for such research use provided that at the time of transfer a copy of this label license is given to the recipients and the recipients agree to be bound by the conditions of this label license. Researchers shall have no right to modify or otherwise create variations of the nucleotide sequence of the luciferase gene or Monster Green® gene except that Researchers may: (1) clone heterologous DNA sequences at either or both ends of said luciferase or Monster Green® gene so as to create fused gene sequences provided that the coding sequence of the resulting luciferase or Monster Green® gene has no more than four deoxynucleotides missing at the affected terminus when compared to the intact luciferase or Monster Green® gene sequence, and (2) insert and remove nucleic acid sequences in furtherance of splicing research predicated on the inactivation or reconstitution of the luminescent activity of the encoded luciferase. In addition, Researchers must do one of the following: (1) use luminescent assay reagents purchased from Promega Corporation for all determinations of luminescence activity resulting from the research use of this product and its derivatives; or, (2) contact Promega Corporation to obtain a license for the use of the product and its derivatives. No other use or transfer of this product or its derivatives is authorized without the express written consent of Promega Corporation including, without limitation, Commercial Use. Commercial Use means any and all uses of this product and derivatives by a party for monetary or other consideration and may include, but is not limited to use in: (1) product manufacture; and (2) to provide a service, information or data; and/or resale of the product or its derivatives, whether or not such product or derivatives are resold for use in research. With respect to such Commercial Use, or any diagnostic, therapeutic or prophylactic uses, please contact Promega Corporation for supply and licensing information. If the purchaser is not willing to accept the conditions of this limited use statement, SABiosciences is willing to accept the return of the unopened product and provide the purchaser with a full refund. However, in the event the product is opened, then the purchaser agrees to be bound by the conditions of this limited use statement. The above license relates to Promega Corporation patents and/or patent applications on improvements to the luciferase and Monster Green® gene.

NOTICE TO PURCHASER I

This product is intended for research purposes only and is not intended for drug or diagnostic purposes or for human use. The purchase of Signal Reporter Assay kits includes a limited, nonexclusive license to use the kit components for research use only. This license does not grant rights to use the kit components for reproduction of any constructs, to modify kit components for resale or to manufacture commercial products without written approval of SABiosciences Corporation. No other license, expressed, implied or by estoppel, is granted.

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Signal Reporter Assays

Signal™ Reporter Assay Kits

Part #1030A

Version 1.8

1/10/2011



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