

TECHNICAL NOTE

Cignal™ Reporter Assay Kit: A High Performance Tool for Assessing the Functions of Genes, Biologics and Small Molecule Compounds

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ABSTRACT

The function and activity of gene products and small molecule drug candidates are often mediated by transcription factors regulated by various signal transduction pathways. Monitoring the activities of these downstream transcription factors is a reliable and proven method for studying the regulation of signaling pathways. The Cignal Reporter Assay Kits are designed to provide a rapid, sensitive, and quantitative assessment of signal transduction pathway activation by measuring the activities of downstream transcription factors. This paper describes the development of these pathway-focused transcription factor-responsive reporter assays. The assays, based on a dual-luciferase technology, generate exceptionally reproducible and reliable results. Every reporter assay is individually engineered to exhibit outstanding sensitivity, specificity, and signal-to-noise ratio. Application examples utilizing RNA interference, gene overexpression, protein treatment, and small molecule compound treatment in combination with the Cignal Reporter Assays are described. Cignal Reporter Assays are a powerful tool for deciphering gene function, as well as determining the mechanisms of action of proteins, peptides, ligands, and small molecule compounds.

INTRODUCTION

The cell-based assay provides an important tool to investigate the biological functions of genes, natural products, or synthetic small molecule compounds under physiological conditions. Transcription factors play a central role in the orchestration of cellular processes including cell development, cell growth, and differentiation. Transcription factors are associated with many human diseases. The activity of transcription factors can also be used as a read-out for the intracellular status of many signal transduction pathways. Thus, the development of reliable cell-based assays designed to measure the activity of transcription factors is a much-needed technology for carrying out fast and efficient global functional genomics and chemical genetics studies. Products for monitoring transcription factor activity have been commercially available. However, those products have suffered from performance shortcomings in one or more areas. Critical features include the sensitivity, specificity, reproducibility, and simplicity of these assays, in addition to the breadth of biological pathway activities that can be measured by a given product line. The **Cignal Reporter Assay Kits** provide an unparalleled combination of high performance, convenience, and breadth of coverage. These assays have been shown to be extremely valuable cell-based assay tools.

We have developed eighteen (18) Cignal Reporter Assay Kits for rapid and sensitive interrogation of transcription factor activities. These cellular assays rely on dual-luciferase reporter technology and cover a wide range of cell signaling pathways. The Cignal Reporter Assays utilize the luciferase system because of its high sensitivity and wide dynamic range. Our pathway-focused transcription factor-responsive firefly luciferase reporters

consist of a combination of specific transcription factor binding sites and basic promoter elements that drive the expression of a luciferase gene. The modulation of a signal transduction pathway results in a change in the activity of a downstream transcription factor. This alteration in transcription factor activity affects its ability to bind a specific DNA target sequence, which in turn changes the expression level of luciferase enzyme. A change in the expression of luciferase enzyme can be easily monitored by a change in luminescence intensity.

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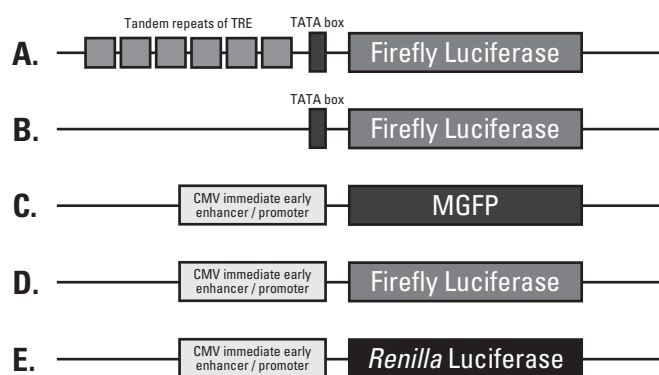


Figure 1: Schematic representation of constructs included in Cignal Reporter Assay Kit.

(A) A pathway-focused transcription factor responsive firefly luciferase reporter, (B) A non-inducible firefly luciferase reporter as a negative control, (C) A constitutively expressing GFP construct, (D) A constitutively expressing firefly luciferase construct, (E) A constitutively expressing *Renilla* luciferase construct.

Each Cignal Reporter Assay kit is specific for a particular transcription factor and/or signal transduction pathway, and includes three components: (1) a pathway-focused transcription factor-responsive firefly luciferase reporter which encodes the firefly luciferase reporter gene under the control of a basal promoter element (TATA box) joined to tandem repeats of a proprietary Transcriptional Response Element (TRE; Fig. 1A); (2) a non-inducible firefly luciferase which encodes firefly luciferase protein under the control of a basal promoter element (TATA box), without any additional transcriptional response elements serves as a negative control (Fig. 1B); (3) a mixture of constitutively expressing GFP and firefly luciferase as a positive control (Fig. 1C, D). To attain more reliable assay results, each of the three kit components have been pre-mixed with a constitutively expressing *Renilla* luciferase reporter (Fig. 1E, Fig. 2).

The use of a single luciferase reporter cannot distinguish between changes in luciferase expression due to changes in the specific transcriptional event under study, or changes due to global and/or technical variability. Therefore the Cignal Reporter Assays utilize dual-luciferase reporter technology to overcome this problem. The dual-luciferase reporter assay system includes two different luciferase reporter enzymes (the firefly luciferase and the *Renilla* luciferase) that are expressed simultaneously in each cell. The firefly luciferase and the *Renilla* luciferase only act upon their respective bioluminescent substrates. The firefly luciferase reporter acts as an experimental reporter and its activity is correlated with the effect of specifically designed experimental conditions. The constitutively expressing *Renilla* luciferase acts as an internal control and provides precise and accurate results by normalizing for unwanted variability caused by well-to-well or plate-to-plate differences in cytotoxicity, transfection efficiency, technical variability, and off-target effects.

In this report, we investigated the properties of the Cignal Reporter Assay Kits and studied their application in functional genomics and chemical genetics.

MATERIALS AND METHODS

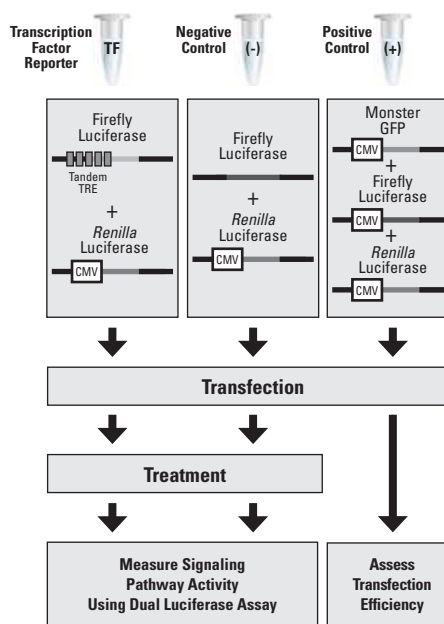


Figure 2: Overview of the Cignal Reporter Assay procedure.

Cignal Reporter Assay

An overview of the Cignal Reporter Assay procedure is depicted in Figure 2. Briefly, the transcription factor-responsive reporter, negative control, and positive control constructs were diluted in Opti-MEM® (Invitrogen) along with the relevant test nucleic acids (siRNA, shRNA, miRNA, expression vector). The diluted nucleic acids were mixed with the diluted SureFECT™ (transfection reagent; SABiosciences Corp.) and delivered to 20,000 cells in a 96-well plate format. Culture media were changed 16-24 hours after transfection. Transfection efficiency was estimated by following the expression of GFP (in the positive control wells) using fluorescence microscopy. Depending upon the experimental parameters, the transfected cells were treated with test proteins, peptides, or compounds of interest. Finally, after an appropriate time of treatment, cells were harvested into cell lysis buffer (Promega). Luciferase activities were determined using the Dual-Luciferase® Assay System (Promega) and an Envision® 2130 multilabel reader (Perkin Elmer). The firefly/*Renilla* activity ratio generated from the transcription factor-responsive reporter transfections was divided by the firefly/*Renilla* activity ratio generated from the negative control transfections to obtain the relative luciferase units. At least three independent transfections were carried out in triplicate for each of the conditions tested with each reporter assay.

Cells and Material Used

ATTC was the supplier of HeLa, CHO-K1, MCF-7, and the HepG2 cell lines. The 293-H cell line was purchased from Invitrogen. CHO-K1 cells were cultured in Ham's F12K medium. All other cell lines were cultured in DMEM. All cell cultures were maintained in growth medium containing 10% FBS, 1X non-essential amino acids (NEAA), penicillin, and streptomycin.

Reporter Constructs

A Cignal Reporter construct (Fig. 1A) consists of three components: (1) the transcriptional regulatory region, (2) a minimal promoter, and (3) a reporter gene. The proprietary combination of these three components yields excellent sensitivity and specificity, enhanced versatility, minimal anomalous background transcription, an excellent signal-to-noise ratio, and a rapid signal response.

RESULTS

Excellent Sensitivity and Specificity

Each Cignal Reporter construct contains a unique and specific transcriptional regulatory region, which consists of tandem repeats of a consensus transcription factor binding site, also referred to as the transcriptional response element (TRE). The sequence of the TRE for any transcription factor varies to some degree from one endogenous promoter to another as well as from one experimental system to another. Therefore, we have used a consensus sequence for each transcription factor binding site to construct each reporter. The specific consensus sequence of each TRE was derived from the published literature. We have optimized the number of response element repeats to maximize the sensitivity and specificity of each assay. Figure 3 shows the results of such an optimization study, for the p53 reporter assay. We designed p53-responsive reporters containing different numbers of p53 binding sites. Although these reporters showed similar basal activities, the final, optimized reporter exhibited much higher sensitivity, and was the construct included in the Cignal p53 Reporter Assay Kit (Fig. 3).

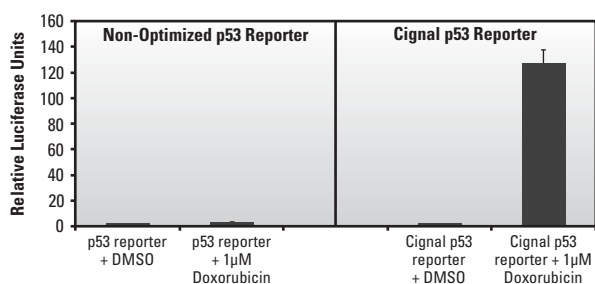


Figure 3. Optimized number of TRE repeats results in excellent sensitivity and specificity for Cignal p53 Reporter Assay.

Transfections were carried out in 293-H cells with either the p53 reporter carrying a suboptimal number of TRE repeats (A) or with the final Cignal p53 Reporter (B). After 16 hours of transfection, medium was changed to assay medium (Opti-MEM + 0.5% FBS + 0.1mM NEAA + 1mM Sodium pyruvate + 100 U/mL penicillin + 100 µg/mL streptomycin). After 24 hours of transfection, cells were treated with 1µM doxorubicin or DMSO alone. Dual-Luciferase assay was performed 18 hours after treatment, and promoter activity values are expressed as arbitrary units using a Renilla reporter for internal normalization. Experiments were done in triplicates, and the standard deviation is indicated.

Maximal Signal-to-Noise Ratio

Next, we determined the best intervening sequence to place between each individual TRE, in order to maximize the signal-to-noise ratio for each reporter. For example, two cAMP response element (CRE)-reporters were designed; one with direct repeats of the CRE sequence (TGACGTCA) and another with the CRE repeats containing our experimentally optimized intervening sequence between each CRE repeat. The cell-based assay demonstrated that the CRE-reporter containing the proprietary intervening sequence between the CRE repeats (Cignal CRE Reporter; Fig. 4) showed better induction with forskolin (a chemical known to elevate the intracellular level of cAMP) as compared to CRE-reporter containing only direct repeats of the CRE (CRE-reporter-1; Fig. 4).

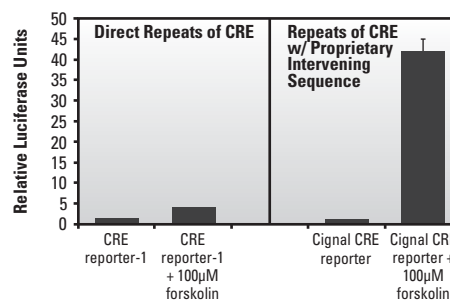


Figure 4. Optimized intervening sequence provides excellent signal-to-noise ratio for Cignal CRE Reporter Assay.

Transfections were carried out in 293-H cells with either CRE reporter containing direct repeats of the CRE sequence (CRE reporter-1) or with the Cignal CRE Reporter containing the same number of CRE repeats along with the proprietary intervening sequence between each repeat. After 16 hours of transfection, cells were treated with 100µM forskolin for 6 hours. Dual-Luciferase assay was performed, and promoter activity values are expressed as arbitrary units using a Renilla reporter for internal normalization. Experiments were done in triplicates, and the standard deviations are indicated.

Minimal Background Transcription and Rapid Response

Cignal Reporter Assays use luciferase as a reporter system because of its high sensitivity, wide dynamic range, and the minimal toxicity of the enzyme even when expressed at high levels. To improve the performance of the assay, we have selected a mammalian codon-optimized (for better expression), non-secreted form of the firefly luciferase gene. The luciferase gene is specifically engineered to minimize the occurrence of cryptic TRE sequences and thereby reduce non-specific expression. Additionally, the selected luciferase gene carries a protein-destabilizing sequence. The cells rapidly degrade the destabilized form of the luciferase protein and hence the background luciferase activity (noise level) is greatly reduced. Due to low background activity, the magnitude of the response that can be measured (signal-to-noise ratio) as well as the speed at which changes in transcription can be measured are enhanced. Thus, the Cignal Reporters yield minimal anomalous background transcription, an excellent signal-to-noise ratio, and a rapid response to regulators of reporter gene transcription. These features were highlighted in a representative study showing the induction of NFκB signaling by recombinant human tumor necrosis factor alpha (hTNFα) protein. The Cignal NFκB Reporter with the destabilized luciferase gene showed reduced basal non-induced activity (noise level; Fig 5A) and hence provided enhanced fold induction (signal-to-noise ratio; Fig 5B), as compared to an NFκB reporter expressing the stable luciferase gene.

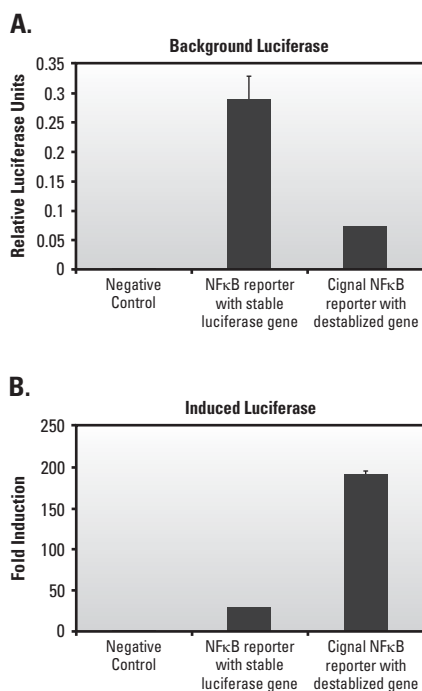


Figure 5. The destabilized luciferase as a reporter gene gives better signal-to-noise ratio.

(A-B) Transfections were carried out in 293-H cells with either NFκB reporter expressing the stable luciferase gene or Cignal NFκB Reporter expressing the destabilized luciferase gene or negative control or positive control. After 16 hours of transfection, medium was changed to assay medium (Opti-MEM + 0.5% FBS + 0.1mM NEAA + 1mM Sodium pyruvate + 100 U/mL penicillin + 100 µg/mL streptomycin). After 24 hours of transfection, cells were either treated with 50 ng/mL of recombinant human tumor necrosis factor alpha (hTNFα) for 6 hours, or were left untreated. Dual-Luciferase assay was performed. (A) The basal non-induced promoter activity values are expressed as arbitrary units using a Renilla reporter for internal normalization. (B) The TNFα-induced promoter activity values are expressed as fold induction (signal-to-noise ratio). Experiments were done in triplicates, and the standard deviation is indicated.

Reduced Experimental Variation and Greater Reliability

The luciferase reporter assay is a convenient method for the quantification of changes in transcription factor activities and their upstream cell signaling pathways. The single luciferase assay, however, does not consider the variabilities that can undermine experimental accuracy, such as differences in the efficiency of cell transfection, cell numbers and cytotoxicity. These deficiencies can be overcome by using a dual-luciferase assay. We have included a constitutively active *Renilla* luciferase (Fig. 1E) with our firefly reporter, negative control and positive control in the Cignal Reporter Assay kits (Fig. 2). The inclusion of *Renilla* luciferase enables the users to obtain reliable, reproducible and accurate results, utilizing a dual-luciferase assay format. To confirm this, a study was carried out to demonstrate the negative regulation of NFκB signaling caused by knocking down the expression of NFκB1 and RelA (Fig. 6). The use of a single luciferase assay, in which only the firefly luciferase levels were monitored, resulted in a huge variation among the experimental triplicates, producing non-conclusive results. The use of the Cignal NFκB Reporter Assay (dual-luciferase assay) reduced the experimental variation and provided more reliable and conclusive results (Fig. 6).

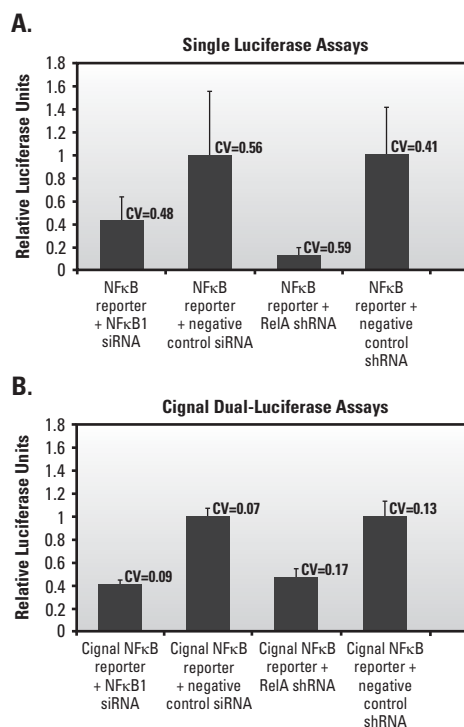


Figure 6. The dual-luciferase assay provides reliable and accurate results.

(A-B) Transfections were carried out in 293-H cells with NFκB reporter construct or with Cignal NFκB Reporter along with NFκB1 siRNA, RelA shRNA, negative control siRNA and negative control shRNA. After 16 hours of transfection, medium was changed to complete growth medium. Experiments were done in triplicates. (A) The single luciferase assay was performed 72 hours after transfection and promoter activity values are expressed as arbitrary units. (B) The dual-luciferase assay was performed 72 hours after the transfection, and promoter activity values are expressed as arbitrary units using a *Renilla* reporter for internal normalization.

Performance Data

We have developed eighteen (18) Cignal Reporter Assay kits. Each kit is designed to monitor the change in the activity (both up- and down-regulation) of a specific transcription factor and its corresponding signaling pathway. These kits were functionally validated by transient transfection of mammalian cell lines. Each reporter reliably quantifies the change in activity of a specific transcription factor and its associated signal transduction pathway in response to treatment with a relevant inducer (Table 2). For example, TCF/LEF reporter, a reporter of Wnt signaling, was transiently transfected into 293-H cells. The transfected 293-H cells were treated with 400 ng/mL of recombinant mouse Wnt3a protein (mWnt3a). After stimulation, both the treated cells and untreated cells were subjected to the dual-luciferase assay. The firefly/*Renilla* activity ratio generated from the treated cells was divided by that from the untreated cells to obtain the 9-fold induction listed for the TCF/LEF Cignal Reporter in Table 2.

Table 1: Performance Data for the Cignal Reporter Assay kits.

Cignal Reporter Assay Kit (Pathway)	Cell Line	Stimulus (Final Concentration)	Fold Induction
C/EBP reporter (C/EBP pathway)	293-H	LiCl (10nM)	10.5
CRE reporter (cAMP/PKA pathway)	293-H	Forskolin (10µM)	213.3
E2F reporter (Cell cycle control)	293-H	Serum (10%) + EGF (100 ng/mL)	26.8
p53 reporter (DNA repair)	293-H	Nutlin-3 (100µM)	117
ERE reporter (Estrogen receptor signaling)	MCF-7	17β-estradiol (E2) (10nM)	2.5
GRE receptor (Glucocorticoid receptor signaling)	HeLa	Dexamethasone (100nM)	23.1
HIF reporter (Hypoxia signaling)	HepG2	CoCl2 (250µM)	4
ISRE reporter (Type I interferon signaling)	HeLa	Recombinant human IFN-α protein (1000 U/mL)	7
GAS reporter (Interferon Gamma signaling)	HeLa	Recombinant human IFN-γ (100 ng/mL)	17.5
SRE reporter (MAPK/ERK)	293-H	Serum (10%) + EGF (100 ng/mL)	42.2
AP-1 reporter (MAPK/JNK)	293-H	PMA (10 ng/mL)	42.6
Myc reporter (Myc pathway)	293-H	Recombinant adenovirus expressing c-Myc (10 MOI)	3
NFκB reporter (NFκB signaling)	293-H	Recombinant human TNF-α protein (50 ng/mL)	191.5
RBP-Jκ reporter (Notch signaling)	293-H	Recombinant adenovirus expressing constitutive active intracellular domain of Notch1 (100 MOI)	280
NFAT reporter (PKC/Ca ²⁺ signaling)	293-H	PMA (10 ng/mL) + ionomycin (0.5µM)	12.4
RARE reporter (Retinoic acid receptor signaling)	CHO-K1	All trans retinoic acid (ATRA) (1µM)	14
SMAD reporter (TGFβ signaling)	293-H	Recombinant human TGFβ protein (25 ng/µL)	29.3
TCF/LEF reporter (Wnt signaling)	293-H	Recombinant mouse Wnt 3a protein (400 ng/mL)	9

Applications

The Cignal Reporter Assay Kits are very useful for studying the phenotypic impact of siRNA / shRNA / miRNA; the functional analysis of the overexpression of genes; the impact of interfering peptides and recombinant protein; and the effect of small chemical molecules or drug candidates in a cell-based assay format. We have performed a case study to show the application of the Cignal p53 Reporter Assay kit to elucidate the biological effect of Dicer siRNA. We have also carried out an overexpression case study in which the effect of Notch1 on p53 transcriptional activity was measured.

Functional Genomics: Assessing RNA Interference Phenotypes

Dicer is a ribonuclease in the RNase III family that cleaves double-stranded RNA (dsRNA) and into two classes of smaller RNA, miRNA and siRNA, which are 20-25 nucleotides in length. MicroRNA (miRNA) repress translation, while siRNA molecules target the selective destruction of homologous RNAs. Dicer mutants are defective for both transcript destruction and translational repression, suggesting that Dicer is required in both the siRNA and miRNA pathways. The phenotypic effect of Dicer knock down on p53 signaling, however, is not known. The Cignal p53 Reporter Assay kit was utilized to understand the biological effect of Dicer siRNA on p53 signaling. The knock down of Dicer using Dicer-specific siRNA was shown to down-regulate p53 signaling (Fig. 7). The data suggest that regulation of p53 signaling is tightly controlled by microRNA and/or siRNA processing.

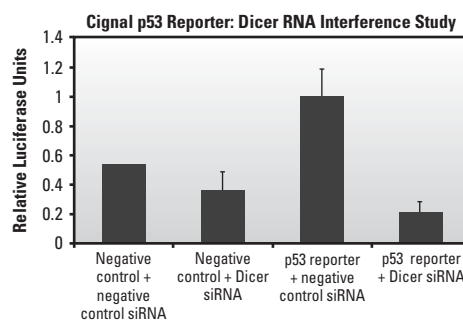


Figure 7. Cignal p53 Reporter Assays showed that Dicer siRNA treatment negatively regulates p53 transcriptional activity.

Transfections were carried out in 293-H cells with Cignal p53 Reporter, negative control and positive control along with Dicer siRNA or negative control siRNA. Dual-Luciferase assays were performed, and promoter activity values are expressed as arbitrary units using a Renilla reporter for internal normalization. Experiments were done in triplicates, and the standard deviation is indicated.

Functional Genomics - Assessing Overexpression Phenotypes

Notch signaling is an important form of intercellular communication and plays a key role in cell fate determination and differentiation. The Notch gene family encodes evolutionarily conserved type 1 transmembrane receptors. The activity of the Notch pathway is critically dependent on context-specific interactions with other signaling pathways. To understand the interaction of Notch signaling with p53 signaling in 293-H cells, we utilized the Cignal p53 Reporter Assay to study the effect of overexpression of constitutively active Notch1 [using recombinant adenoviruses expressing constitutively active Notch1 (Ad-NICD)] on p53 signaling. The results showed that overexpression of constitutively active Notch1 activates the p53 signaling pathway, leading to an induction of p53 transcriptional activity (Fig. 8). This suggests that Notch signaling positively regulates the p53 signaling pathway in 293-H cells.

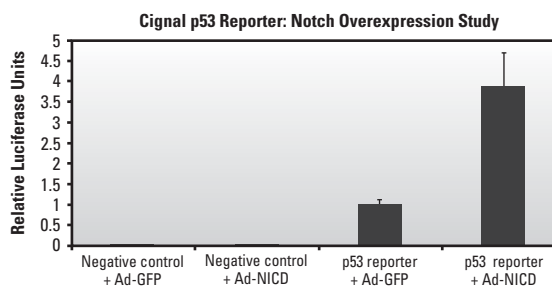


Figure 8. Cignal p53 Reporter Assays showed that Notch signaling positively regulates p53 signaling.

Transfections were carried out in 293-H cells with Cignal p53 Reporter, negative control and positive control. After 24 hours of transfection, cells were infected with 100 MOI of recombinant adenovirus expressing constitutively active Notch1 (Ad-NICD) or 100 MOI of recombinant adenovirus expressing GFP (Ad-GFP). Dual-Luciferase assay was performed, and promoter activity values are expressed as arbitrary units using a Renilla reporter for internal normalization. Experiments were done in triplicates, and the standard deviation is indicated.

DISCUSSION

The cell-based reporter assay has been commonly used in the study of transcriptional regulation. The pathway-focused Signal Reporter Assays are an invaluable tool to monitor changes in the activities of signaling pathways. The Signal Reporter Assay kits cover a wide range of biological pathways such as **cancer biology**: Notch, Wnt/ β -Catenin, TGF β , p53, HIF, Myc, E2F, NF κ B signaling; **cell cycle control**: E2F, p53, Myc; **immunology**: NF κ B, NFAT, Type 1 IFN, INF γ signaling; **cell proliferation**: MAPK/JNK (AP-1), MAPK/ERK (SRE), C/EBP, Myc; **developmental biology**: Notch, Wnt/ β -Catenin, TGF β ; **nuclear hormone receptor biology**: estrogen receptor, glucocorticoid receptor and retinoic acid receptor; **hypoxia signaling**: HIF, p53, E2F, Myc; **GPCR signaling**: MAPK/ERK, NFAT, CRE; **PKC/Ca²⁺ signaling**; and **cAMP/PKA signaling**.

The Signal reporter constructs are extensively engineered to minimize anomalous transcription and background activity. They have also been designed to maximize the specificity, sensitivity, signal-to-noise ratio, and the speed of measuring changes in transcription. The proprietary design of each TRE, coupled with the broad dynamic range of the luciferase assay system, provides exceptional versatility to the Signal Reporter Assays. Researchers can reliably monitor both up- and down-regulation of signaling activities. For example, as shown in figure 5 and 6, the Signal NF κ B Reporter Assay can monitor both the up-regulation of NF κ B signaling by hTNF α protein as well as the down-regulation of NF κ B signaling by RelA shRNA and NF κ B siRNA.

The Signal Reporter Assay kits have the unique advantage of offering ready-to-transfect constructs. This provides the reliability and convenience of carrying out experiments directly using the kit components, eliminating the time-consuming tasks of construct amplifications and purifications.

Our application results have confirmed that the Signal Reporter Assay kits serve as outstanding tools for carrying out functional genomics studies to assess the biological impact of siRNA, shRNA, and cDNA. In addition, the kits will accelerate the speed at which researchers elucidate the physiological functions and off-target effects of small chemical molecules and recombinant proteins.

Taken together, the Signal Reporter Assays provide outstanding sensitivity, reproducibility, versatility, and convenience for carrying out a quantitative assessment of the regulation of signal transduction pathways.

APPENDIX

High Efficiency, Low Toxicity Reverse Transfection with the SureFECT™ Transfection Reagent

Reverse transfection saves an entire day over traditional transfection by plating cells directly into wells and medium already containing reagent-DNA complexes (Fig. 9). Reverse transfection produces equivalent or improved transfection efficiencies and reproducibility over standard pre-plated methods. The SureFECT Transfection Reagent has been carefully engineered to provide maximal reverse transfection efficiencies.

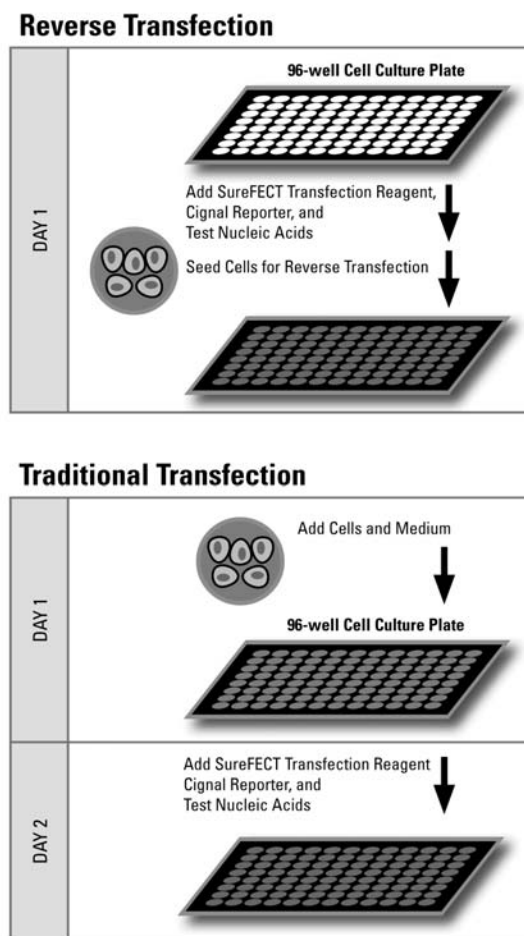


Figure 9. Reverse Transfection vs. Traditional Transfection Protocol Workflow

The success of any cell-based assay that involves transient transfection is dependent upon the transfection efficiency and cytotoxicity associated with the transfection protocol used in the experiment. Developing an optimized transfection protocol for a given cell line, where maximal transfection efficiency and minimal cytotoxicity occurs, can be a time consuming and tedious process. All of the data presented in this white paper came from experiments utilizing the SureFECT transfection reagent for the reverse transfection of cells grown in 96-well cell culture plates. Reviewing Table 1 confirms that the SureFECT transfection reagent works well with numerous cell types. The SureFECT reagent has also been successfully used in several other cell lines. These include HCT1080, HCT116, Cos7, MDA-MB231, A549, PC3, CV1, and BHK cell lines. In addition to its utility with numerous cell lines, the SureFECT reverse transfection protocol results in superior transfection efficiencies (Fig. 10) and cell viabilities (Fig. 11), when compared to alternative transfection reagents.

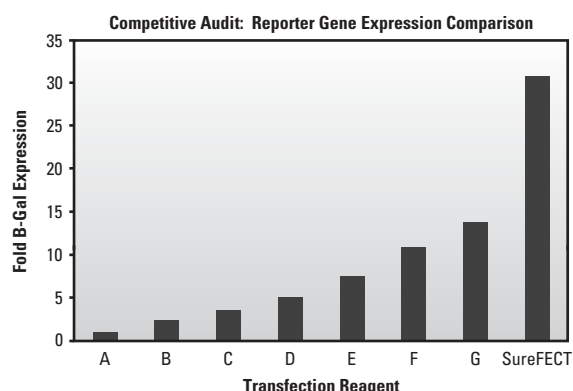


Figure 10. SureFECT Reverse Transfects with Superior Efficiency to Traditional Transfection Reagents.

In 96-well plates, 50 μ L of diluted DNA (0.33 μ g pCMV β -Gal plasmid) was mixed with 50 μ L dilutions of seven different commercial transfection reagents in serum-free Opti-MEM. COS7 cells (15,000 in 50 μ L) in normal medium containing 5% FBS were then added to the wells for reverse transfection. Enzyme activity (OD570) was assayed 24 h after transfection with 0.5 mg/mL CRPG substrate in assay buffer.

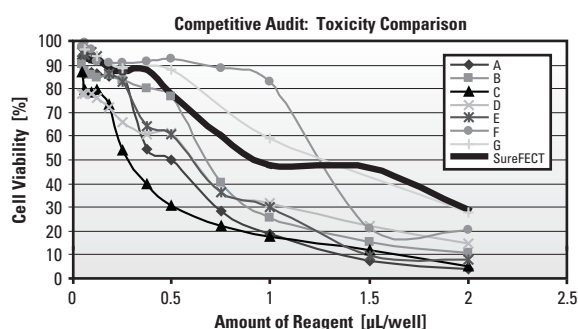


Figure 11. SureFECT Maintains the Viability of Efficiently Reverse Transfected Cells.

In 96-well plates, 50 μ L of diluted DNA (0.33 μ g pCMV β -Gal plasmid) was mixed with 50 μ L dilutions containing four different amounts of eight different commercial transfection reagents in serum-free Opti-MEM. COS7 cells (15,000 in 50 μ L) in normal medium containing 5% FBS were then added to the wells for reverse transfection. Media were changed 24 h post-transfection. Viability was measured 32 h post-transfection utilizing an acidic phosphatase assay.

Most transfection reagents that work well for the delivery of plasmids, do not work well for siRNA delivery, and vice versa. The SureFECT transfection reagent is very unique in this regard. It has been engineered to be an extremely versatile reagent that works well for the transfection of both plasmids and siRNA. SureFECT performs exceptionally well in siRNA transfections on a wide range of cell lines, even those that are known to be difficult to transfect with plasmids (Fig. 12).

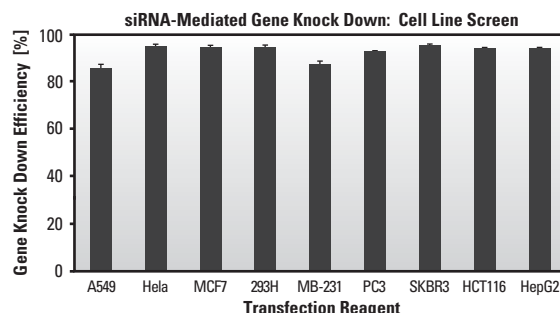


Figure 12: The SureFECT transfection reagent works exceptionally well for the reverse transfection of all mammalian cell lines tested to date.

SureFECT (0.3 μ L per well) was used to reverse transfect MAPK1 siRNA (2 pmole) into different cell types in a 96-well plate. MAPK1 mRNA levels were measured 48 h after transfection using quantitative real-time RT-PCR. The knock down efficiency (versus a negative control siRNA) is calculated via the $\Delta\Delta C_t$ method.

Signal Reporter Assay Kits

Pathway	Transcriptional Regulatory Element (TRE)	Transcription Factor	Catalog Number
C/EBP reporter	C/EBP binding element	C/EBP	CCS-001L
cAMP/PKA	cAMP regulatory element (CRE)	CREB	CCS-002L
Cell Cycle	E2F binding element	E2F/DP1	CCS-003L
DNA Damage	p53 response element	p53	CCS-004L
Estrogen Receptor	Estrogen response element (ERE)	Estrogen Receptor (ER)	CCS-005L
Glucocorticoid Receptor	Glucocorticoid response element (GRE)	Glucocorticoid Receptor (GR)	CCS-006L
Hypoxia	HIF response element	Hypoxia-inducible factor-1 (HIF-1)	CCS-007L
Type I Interferon	Interferon stimulated response element (ISRE)	STAT1/STAT2	CCS-008L
Interferon Gamma	Interferon gamma activation sequence (GAS)	STAT1/STAT1	CCS-009L
MAPK/ERK	Serum response element (SRE)	EIK-1/SRF	CCS-010L
MAPK/JNK	AP-1 binding element	AP-1	CCS-011L
c-Myc	E-box binding element	Myc/Max	CCS-012L
NF κ B	NF κ B binding element	NF κ B	CCS-013L
Notch	RBP-Jk binding element	RBP-Jk	CCS-014L
PKC/Ca ²⁺	NFAT binding element	NFAT	CCS-015L
Retinoic Acid Receptor	Retinoic acid response element (RARE)	Retinoic Acid Receptor (RAR)	CCS-016L
TGF β	SMAD response element	SMAD2/SMAD3/SMAD4	CCS-017L
Wnt	TCF/LEF response element	TCF/LEF	CCS-018L

Product	Total Volume	Catalog Number
SureFECT Transfection Reagent	0.5 mL (enough for 1470 reporter assays - 15 x 96 well plates)	SA-01

Signal Reporter Assay Kit Specifications

Component	Specification	Concentration & Total Volume
Reporter	A mixture of an inducible transcription factor responsive firefly luciferase reporter and constitutively expressing Renilla construct (40:1)	(100 ng/ μ L; 500 μ L)
Negative Control	A mixture of non-inducible firefly luciferase reporter and constitutively expressing Renilla construct (40:1)	(100 ng/ μ L; 500 μ L)
Positive Control	A mixture of constitutively expressing GFP construct, constitutively expressing firefly luciferase construct, and constitutively expressing Renilla 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.

