Scientific article

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 are often mediated by transcription factors, whose activities are regulated by signal transduction pathways. Monitoring the activities of downstream transcription factors is a reliable and proven method for studying the regulation of signaling pathways. Cignal Reporter Assay Kits are designed to provide rapid, sensitive, 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. Cignal Reporter Assays are based on dual-luciferase technology, and generate exceptionally reproducible and reliable results. Every reporter assay is individually engineered to exhibit outstanding sensitivity, specificity, and signal-to-noise ratio. Cignal Reporter Assays can be used for a range of applications, including RNA interference, gene overexpression, protein treatment, and small-molecule compound treatment, and examples for each of these applications are presented. These assays can be used for virtually any mammalian cell, and are available as either transfection-ready DNA-based vectors or transduction-ready lentiviruses. In summary, Cignal Reporter Assays are a powerful, cell-based tool for deciphering gene function, as well as determining the mechanism of action for proteins, peptides, ligands, and small-molecule compounds.

Introduction

Cell-based assays provide an important tool to investigate the biological effects of genes, natural products, and synthetic small-molecule compounds under physiological conditions. Transcription factors play a central role in regulating gene expression, orchestrating a host of cellular processes, and are associated with many human diseases. Transcription factor activity can also be used as a readout for the activation status of signal transduction pathways. Therefore, the development of reliable cell-based assays to measure transcription factor activity is a crucial technology for fast and efficient global functional genomics and chemical genetics studies. Optimally, such assays will allow the study of a wide breadth of biological pathways in addition to providing sensitivity, specificity, reproducibility, and simplicity. However, other technologies have not yet met all of these criteria. Cignal Reporter Assay Kits provide an unparalleled combination of high performance, convenience, and breadth of biological pathway coverage, making them valuable cell-based tools for studying signaling pathway activity.

Contents

Forty-five Cignal Reporter Assay Kits have been developed for rapid and sensitive interrogation of transcription factor activities in a wide range of cell signaling pathways. These cellular assays rely on dual-luciferase reporter technology, which provides high sensitivity and a wide dynamic range. These pathway-focused, transcription factor-responsive firefly luciferase reporters comprise a combination of specific transcription factor binding sites and basic promoter elements that drive expression of the luciferase gene. When a signal transduction pathway is modulated, changing the activity of an associated downstream transcription factor, the expression level of the luciferase enzyme is altered as well. Elevated or diminished luciferase expression will cause a change in luminescence intensity.

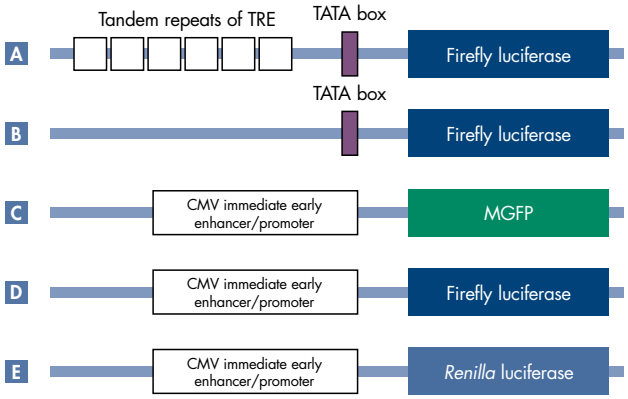

Figure 1. Schematic representation of constructs included in Cignal Reporter Assay Kits. Each kit contains A a pathway-focused transcription factor-responsive firefly luciferase reporter with a variable number of Transcriptional Response Element (TRE) repeats, B a non-inducible firefly luciferase reporter as a negative control, C a constitutively expressing GFP construct containing Monster Green® Fluorescent Protein (MGFP), D a constitutively expressing firefly luciferase construct, and 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 3 components. The first is a transcription factor-responsive firefly luciferase reporter, which encodes the luciferase reporter gene under the control of a basal promoter element, the TATA box, joined to tandem repeats of a proprietary Transcriptional Response Element (TRE, Figure 1A). The second kit component is a negative control, which is a non-inducible firefly luciferase under the control of the TATA box element without addition of TREs (Figure 1B). The third component is the positive control, which is a mixture of constitutively expressing GFP and firefly luciferase (Figure 1C–D).

Single-luciferase reporter assays cannot distinguish between luciferase expression changes that result from the specific transcriptional event under study or changes resulting from global or technical variability. Cignal Reporter Assays overcome this challenge by using dual-luciferase reporter technology. Dual-luciferase reporter technology simultaneously expresses 2 different luciferase reporter enzymes, firefly luciferase and Renilla luciferase, in each cell. Each luciferase will only act upon its own respective bioluminescent substrate. Therefore, the firefly luciferase reporter acts as an experimental reporter, its activity correlating with the effects 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. Each of the 3 components in Cignal Reporter Assay Kits comes pre-mixed with a constitutively expressing Renilla luciferase reporter (Figure 1E, Figure 2), providing more reliable assay results. In this report, we investigated the properties of Cignal Reporter Assay Kits and studied their application in functional genomics and chemical genetics.

Cignal Reporter Assay Kits

Pathway	Transcription factor	Transfection-ready DNA-based vector	Transduction-ready Lentiviral vector
Amino Acid Deprivation	ATF4/ATF3/ATF2	CCS-8034L	CLS-5034L
Androgen	Androgen Receptor	CCS-1019L	CLS-8019L
Antioxidant Response	Nrf2 & Nrf1	CCS-5020L	CLS-2020L
ATF6	ATF6	CCS-9031L	CLS-6031L
C/EBP	C/EBP	CCS-001L	CLS-001L
cAMP/PKA	CREB	CCS-002L	CLS-002L
Cell Cycle	E2F/DP1	CCS-003L	CLS-003L
DNA Damage	p53	CCS-004L	
EGR1	EGR1	CCS-8021L	CLS-5021L
ER Stress	CBF/NF-Y/YY1	CCS-2032L	CLS-9032L
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 Stress	MTF1	CCS-5033L	CLS-2033L
Hedgehog	GLI	CCS-6030L	CLS-3030L
Hepatocyte Nuclear Factor 4	HNF4	CCS-3039L	
Hypoxia	Hypoxia-inducible factor-1 (HIF-1α)	CCS-007L	CLS-007L
Interferon Regulation	IRF1	CCS-7040L	CLS-4040L
Type I Interferon	STAT1/STAT2	CCS-008L	CLS-008L
Interferon Gamma	STAT1/STAT1	CCS-009L	CLS-009L
KLF4	KLF4	CCS-4036L	CLS-1036L
Liver X Receptor	LXRa	CCS-0041L	CLS-7041L
MAPK/ERK	Elk-1/SRF	CCS-010L	CLS-010L
MAPK/JNK	AP-1	CCS-011L	CLS-011L
MEF2	MEF2	CCS-7024L	CLS-4024L
c-myc	Myc/Max	CCS-012L	CLS-012L
Nanog	Nanog	CCS-7037L	CLS-4037L
NFκB	NFκB	CCS-013L	CLS-013L
Notch	RBP-Jk	CCS-014L	CLS-014L
Oct4	Oct4	CCS-0025L	CLS-7025L
Pax6	Pax6	CCS-3042L	
PI3K/AKT	FOXO	CCS-1022L	CLS-8022L
PKC/Ca^{++}	NFAT	CCS-015L	CLS-015L
Peroxisome Proliferator-Activated Receptor (PPAR)	PPAR	CCS-3026L	
Progesterone	Progesterone Receptor (PR)	CCS-6043L	
Retinoic Acid Receptor	Retinoic Acid Receptor (RAR)	CCS-016L	CLS-016L
Retinoid X Receptor	Retinoid X Receptor (RXR)	CCS-9044L	CLS-6044L
Sox2	Sox2	CCS-0038L	
SP1	SP1	CCS-6027L	CLS-3027L
STAT3	STAT3	CCS-9028L	CLS-6028L
TGFβ	SMAD2/SMAD3/SMAD4	CCS-017L	CLS-017L
Vitamin D	Vitamin D Receptor (VDR)	CCS-2029L	CLS-9029L
Wnt	TCF/LEF	CCS-018L	CLS-018L
Xenobiotic	AhR	CCS-2045L	CLS-9045L

Materials and methods

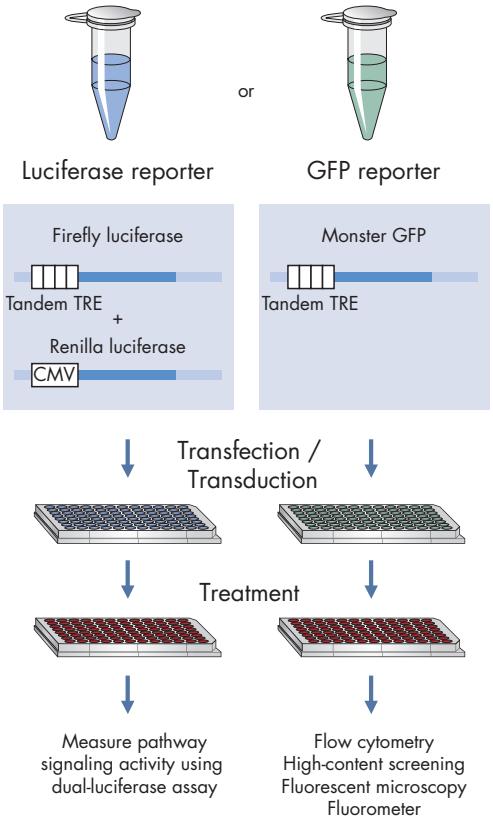

Figure 2. Overview of Cignal Reporter Assay workflow.

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® (Life Technologies, Inc.) along with the relevant test nucleic acids (siRNA, shRNA, miRNA, expression vector). The diluted nucleic acids were mixed with the diluted transfection reagent and delivered to 2 x 10^4 cells in a 96-well plate. Culture media was changed 16–24 hours after transfection. Transfection efficiency was estimated by observing GFP expression in the positive control wells by fluorescence microscopy. Transfected cells were treated with test proteins, peptides, or compounds of interest for an appropriate period of time. Following treatment, cells were harvested into cell lysis buffer (Promega), and luciferase activity was measured using the Dual-Luciferase® Assay System (Promega) and an EnVision® 2103 Multilabel Reader (PerkinElmer). Firefly:Renilla activity ratios were generated from experimental and control transfections. Ratios from transcription factor-responsive reporter transfections were divided by ratios from negative control transfections to obtain relative luciferase units. At least 3 independent transfections were performed in triplicate for each of the conditions tested with each reporter assay.

Cells and materials used

HeLa, CHO-K1, MCF-7, and HepG2 cell lines were obtained from ATCC. The 293-H cell line was purchased from Invitrogen. CHO-K1 cells were cultured in Ham's F12K medium, and 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

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

Cignal Reporter Assay Kit specifications

Component	Specification
Reporter	A mixture of an inducible transcription factor responsive firely luciferase reporter and constitutively expressing Renilla construct (40:1)
Negative control	A mixture of non-inducible firefly luciferase reporter and constitutively expressing Renilla construct (40:1)
Positive control	A mixture of constitutively expressing GFP construct, constitutively expressing firefly luciferase construct, and constitutively expressing Renilla luciferase construct (40:1:1)

Note: These constructs are transfection-grade and are ready for transient transfection.

Results

Excellent sensitivity and specificity

Each Cignal Reporter Assay contains a unique and specific transcriptional regulatory region, which consists of tandem repeats of a consensus transcription factor binding site referred to as the transcriptional response element (TRE). The sequence of the TRE for any transcription factor varies from one endogenous promoter to another, and variation also exists between experimental systems. Therefore, a consensus sequence for each transcription factor binding site was used to construct each reporter. The specific consensus sequence for each TRE was derived from the published literature, and the number of response element repeats was experimentally optimized number to maximize the sensitivity and specificity of each assay. Figure 3 shows the results of such an optimization study for the p53 Cignal Reporter Assay.

Reporters for p53 were designed with different numbers of p53 binding sites. The reporters showed similar basal activities, but the final, optimized reporter exhibited much greater sensitivity, and was therefore included in the p53 Cignal Reporter Assay Kit (Figure 3).

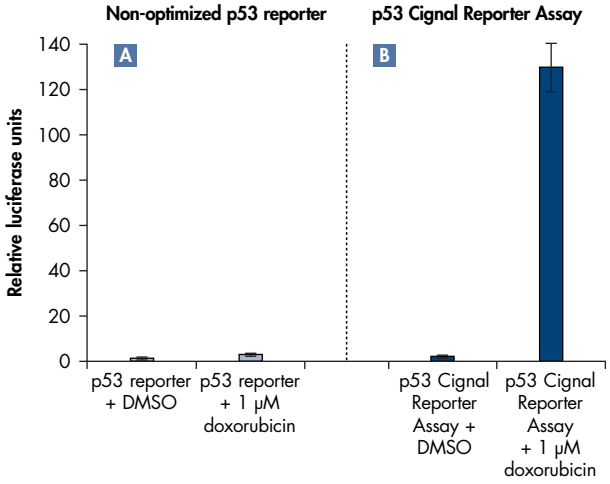

Figure 3. Optimized number of TRE repeats results in excellent sensitivity and specificity for Cignal p53 Reporter Assay. 293-H cells were transfected with either a p53 reporter carrying a suboptimal number of TRE repeats A or with the final p53 Cignal Reporter Assay B. After 16 hours of transfection, medium was changed to assay medium (Opti-MEM + 0.5% FBS + 0.1mM NEAA + 1 mM Sodium pyruvate + 100 U/ml penicillin + 100 µg/ml streptomycin). After 24 hours of transfection, cells were treated with 1 µM doxorubicin or DMSO alone. A 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

We next determined the optimal intervening sequences to place between individual TREs to maximize the signal-to-noise ratio for each reporter. For example, 2 cAMP response element (CRE) reporters were designed, one with direct repeats of the CRE sequence (TGACGTCA) and another with the CRE repeats containing an experimentally optimized intervening sequence between each CRE repeat. The cell-based assay demonstrated that the reporter containing the proprietary intervening sequences between CRE repeats (CRE Cignal Reporter Assay; Figure 4) delivered greater induction with forskolin, which is known to elevate intracellular cAMP, compared to the CRE reporter that contained only direct CRE repeats (CRE-reporter-1; Figure 4).

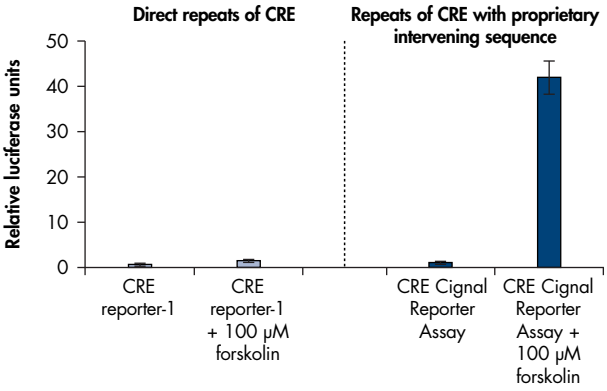

Figure 4. Optimized intervening sequence provides excellent signal-to-noise ratio for CRE Cignal Reporter Assay. 293-H cells were transfected with either CRE reporter containing direct repeats of the CRE sequence (CRE reporter-1) or with the CRE Cignal Reporter Assay containing the same number of CRE repeats along with the proprietary intervening sequence between each repeat. After 16 hours of transfection, cells were treated with 100 µM forskolin for 6 hours. A 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 because of its high sensitivity, wide dynamic range, and low cytotoxicity even at high expression levels. To improve the performance of the assay, we selected a non-secreted form of the firefly luciferase gene optimized for mammalian codons to yield better expression. This luciferase gene is specifically engineered to minimize the occurrence of cryptic TRE sequences and thereby reduce nonspecific expression. The gene also carries a protein-destabilizing sequence. This sequence is crucial because it allows cells to rapidly degrade the destabilized form of the luciferase protein, therefore greatly reducing the background luciferase activity. With low background activity, the signal-to-noise ratio (ie, the magnitude of the response that can be measured) is enhanced, as is the speed at which changes in transcription can be measured. Thus, Cignal Reporter Assays yield minimal anomalous background transcription, an excellent signal-to-noise ratio, and rapid response to regulators of reporter gene transcription. These features were highlighted in a representative study showing induction of NFκB signaling by recombinant human tumor necrosis factor alpha (hTNFα) protein. The NFκB Cignal Reporter Assay with the destabilized luciferase gene showed reduced basal, non-induced activity, or noise (Figure 5A), and hence provided enhanced fold induction, or signal-to-noise ratio (Figure 5B), compared to an NFκB reporter expressing the stable luciferase gene.

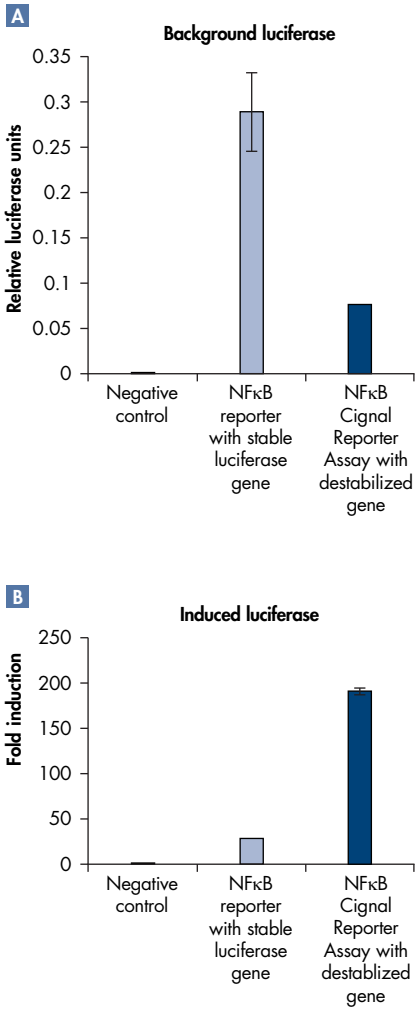

Figure 5. Destabilized luciferase as a reporter gene yields greater signal-to-noise ratio. 293-H cells were transfected with either NFκB reporter expressing the stable luciferase gene or NFκB Cignal Reporter Assay expressing the destabilized luciferase gene, as well as positive or negative controls. 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. A dual-luciferase assay was performed. A Basal non-induced promoter activity values are expressed as arbitrary units using a Renilla reporter for internal normalization. B 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 to quantify activity changes in transcription factors and their upstream signaling pathways. However, the single-luciferase assay does not consider several variables that can undermine experimental accuracy, such as differences in cell number, cytotoxicity, or transfection efficiency. These challenges can be overcome by using a dual-luciferase assay. Cignal Reporter Assay Kits include a constitutively active Renilla luciferase (Figure 1E) with the firefly reporter, negative control, and positive control (Figure 2). This dual-luciferase format yields more reliable, reproducible, and accurate results than the single-luciferase format. To confirm this, we knocked down expression of NFκB1 and RelA to demonstrate negative regulation of NFκB signaling (Figure 6). The single-luciferase assay, monitoring only firefly luciferase levels, yielded huge variation among the triplicate samples, producing inconclusive results. However, the dual-luciferase NFκB Cignal Reporter Assay reduced this experimental variation, providing more reliable and conclusive results (Figure 6).

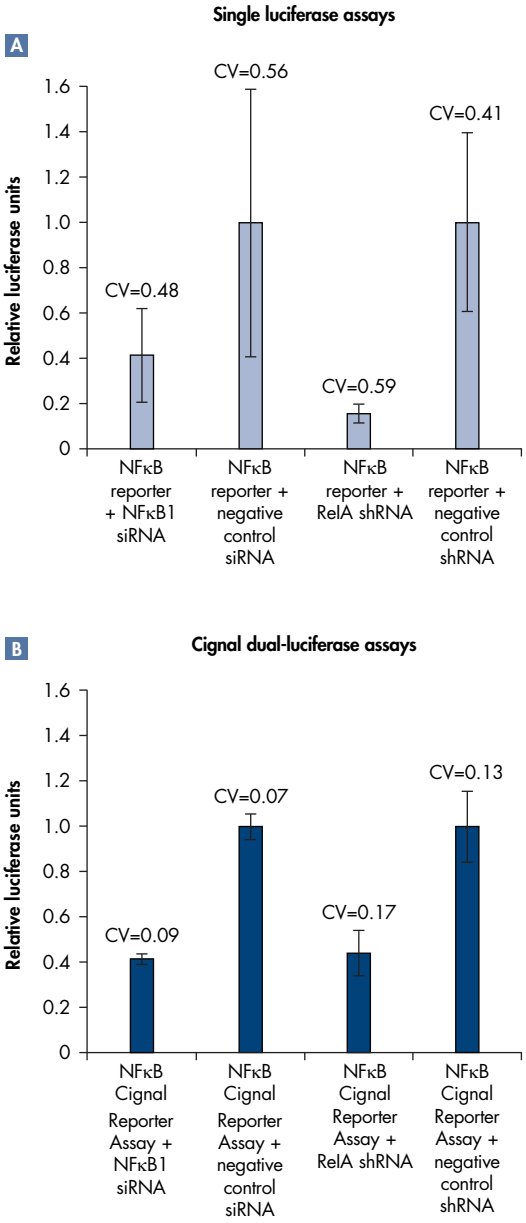

Figure 6. The dual-luciferase assay provides reliable and accurate results. (A-B) 293-H cells were transfected with NFκB reporter construct or with NFκB Cignal Reporter Assay along with NFκB1 siRNA, RelA shRNA, negative control siRNA and negative control shRNA. After 16 hours of transfection, medium 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 transfection, and promoter activity values are expressed as arbitrary units using a Renilla reporter for internal normalization.

Performance data

Each of the 45 Cignal Reporter Assay Kits is designed to monitor the change in activity, both up- and downregulation, of a specific transcription factor and its corresponding signaling pathway. The performance of these kits was functionally verified 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 1). For example, the TCF/LEF Cignal Reporter Assay, a reporter of Wnt signaling, was transiently transfected into 293-H cells. The transfected cells were treated with 400 ng/ml of recombinant mouse Wnt3a protein (mWnt3a). After stimulation, both treated and untreated cells were analyzed by 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 this reporter in Table 1.

Table 1. Performance data for the Cignal Reporter Assay Kits.

Cignal Reporter Assay Kit (Pathway)	Cell line	Stimulus (Final concentration)	Fold induction
AARE reporter (Amino acid deprivation)	HeLa	Leucine starvation	5.9
AR reporter (Androgen pathway)	LNCaP	Mibolerone (3.2 nM)	8.9
ARE reporter (Antioxidant pathway)	HepG2	DL-Sulforaphane (10 µM)	9.4
ATF6 reporter (ATF6 pathway)	HeLa	Thapsigargin (100 nM)	3.8
C/EBP reporter (C/EBP pathway)	293-H	LiCl (10 mM)	10.5
CRE reporter (cAMP/PKA pathway)	293-H	Forskolin (10 µM)	213.3
E2F reporter (Cell cycle control)	293-H	Serum (10%) + EGF (100 ng/mL)	26.8
p53 reporter (DNA damage)	293-H	Nutlin-3 (100 µM)	117
EGR1 reporter (EGR1 pathway)	293-H	PMA (10 ng/ml)	220.7
ERSE reporter (ER stress)	HeLa	Thapsigargin (100 nM)	4.1
ERE reporter (Estrogen receptor signaling)	MCF-7	17b-estradiol (E2) (10 nM)	2.5
GATA reporter (GATA pathway)	HEK-293	PMA (10 ng/ml)	2.9
GR receptor (Glucocorticoid receptor signaling)	HeLa	Dexamethasone (100 nM)	23.1
HSE reporter (Heat shock response)	HeLa	17-AAG (50 nM)	9.3
MTF1 reporter (Heavy metal stress)	HeLa	ZnSO4 (100 µM)	8.7
GLI reporter (Hedgehog signaling)	NIH3T3	CMV-mG1 + mSHH (400 µg/ml)	56.5
HNF4 reporter (HNF4 signaling)	HepG2	Constitutively expressing HNF4 vector	32
HIF-1 reporter (Hypoxia)	HepG2	CoCl2 (250 µM)	26.8
IRF1 (Interferon Regulation)	HeLa	IFN-g (10 ng/ml)	25.9
ISRE reporter (Type I	HeLa	Dexamethasone (100 nM)	23.1
IFN signaling)	HeLa	Recombinant human IFN-alpha protein (1000 U/ml)	7
GAS reporter (IFN-g signaling)	HeLa	Recombinant human IFN-g (100 ng/ml)	17.5
KLF4 reporter (KLF4 signaling)	293-H	CMV-KLF4 expression vector	68
LXRa (LXR signaling)	293-H	LXRa expression vector	238
SRE reporter (MAPK/ERK signaling)	293-H	Serum (10%) + EGF (100 ng/ml)	42.2
AP-1 reporter (MAP/JNK signaling)	293-H	PMA (10 ng/ml)	42.6
MEF2 reporter (MEF2 signaling)	293-H	Adenovirus expressing MEF2 (10 MOI) + PMA (10 ng/ml)	10.5
Myc reporter (Myc pathway)	293-H	Recombinant adenovirus expressing c-Myc (10 MOI)	3
Nanog reporter (Nanog signaling)	293-H	CMV-Nanog expression vector	11.9
NFkB reporter (NFkB signaling)	293-H	Recombinant human TNF-alpha protein (50 ng/ml)	191.5
RBP-Jk reporter (Notch signaling)	293-H	Recombinant adenovirus expressing constitutive active intracellular domain of Notch1 (100 MOI)	280
Oct4 reporter (Oct4 signaling)	293-H	CMV-Oct4 (100 ng)	21.9
Pax6 reporter (Pax6 signaling)	293-H	Constitutively expressing Pax6 expression vector	161
FOXO reporter (PI3K/AKT signaling)	293-H	Ad-FOXO3A (10 MOI)	51.2
NFAT reporter (PKC/Ca++ signaling)	293-H	PMA (10 ng/ml) + ionomycin (0.5 µM)	12.4
PPAR reporter (PPAR signaling)	HepG2	PPAR-g expression vector + 1 µM rosaglitazone	3.3
PR reporter (PR signaling)	HeLa	PR expression vector + progesterone (10 nM)	17
RARE reporter (Retinoic acid receptor signaling)	CHO-K1	All-Trans-Retinoic acid (ATRA) (1 µM)	14
RXR reporter (RXR signaling)	CHO-K1	ATRA (5 µM)	4.1
Sox2 reporter (Sox2 signaling)	293-H	CMV-Sox2 expression vector	12.3
SP1 reporter (SP1 signaling)	HeLa	Trichostatin A (100 ng/ml)	15.6
STAT3 reporter (STAT3 signaling)	HepG2	IL-6 (10 ng/ml)	17.7
SMAD reporter (TGFb signaling)	293-H	Recombinant human TGF-beta protein (25 ng/µl)	29.3
VDRE reporter (Vitamin D receptor signaling)	HeLa	Calcitriol (0.05 µM)	20.5
TCF/LEF reporter (Wnt signaling)	293-H	Recombinant mouse Wnt3a protein (400 ng/ml)	9
XRE reporter (AhR signaling)	HepG2	2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD) (10 nM)	134.2

Applications

Cignal Reporter Assay Kits are useful for studying phenotypic alterations as a result of gene silencing (such as with siRNA, shRNA, or miRNA), functional changes due to gene overexpression, the impact of interfering peptides and recombinant proteins, and the effects of small chemical molecules or drug candidates. We have performed a case study to demonstrate the effectiveness of the p53 Cignal Reporter Assay Kit in elucidating the biological effects of Dicer siRNA. We have also carried out an overexpression case study measuring the effect of Notch1 on p53 transcriptional activity.

Functional genomics: assessing RNA interference phenotypes

Dicer, an RNase III ribonuclease, cleaves double-stranded RNA (dsRNA) into 2 classes of small RNAs about 20–25 nucleotides in length. These are microRNAs (miRNAs), which repress translation, and small interfering RNAs (siRNAs), which target homologous RNAs for selective destruction. Dicer mutants are defective for both transcript destruction and translational repression, suggesting that Dicer is required for both the siRNA and miRNA pathways. The phenotypic effect of Dicer knockdown on p53 signaling, however, is unknown. The p53 Cignal Reporter Assay Kit was used to measure the biological effect of Dicer siRNA on p53 signaling. The knockdown of Dicer using Dicer-specific siRNA was shown to downregulate p53 signaling (Figure 7). These data suggest that regulation of p53 signaling is tightly controlled by miRNA and/or siRNA.

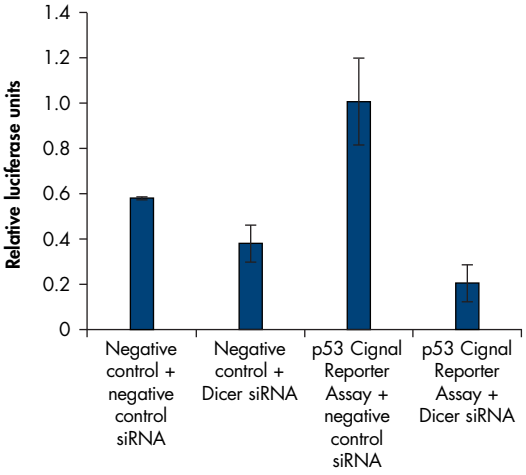

Figure 7. p53 Cignal Reporter Assays show that Dicer siRNA treatment negatively regulates p53 transcriptional activity. 293-H cells were transfected with p53 Cignal Reporter Assay, negative control and positive control along with Dicer siRNA or negative control siRNA. Dual-luciferase assays were performed, 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 mechanism of intercellular communication and plays a key role in cell fate determination and differentiation. The Notch gene family encodes evolutionarily conserved Type I 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 used the p53 Cignal Reporter Assay to study how overexpressing Notch1 affects p53 signaling. To do this, we utilized recombinant adenoviruses expressing constitutively active Notch1 (Ad-NICD). The results showed that overexpression of constitutively active Notch1 activates the p53 signaling pathway, leading to induction of p53 transcriptional activity (Figure 8). This suggests that Notch signaling positively regulates the p53 signaling pathway in 293-H cells.

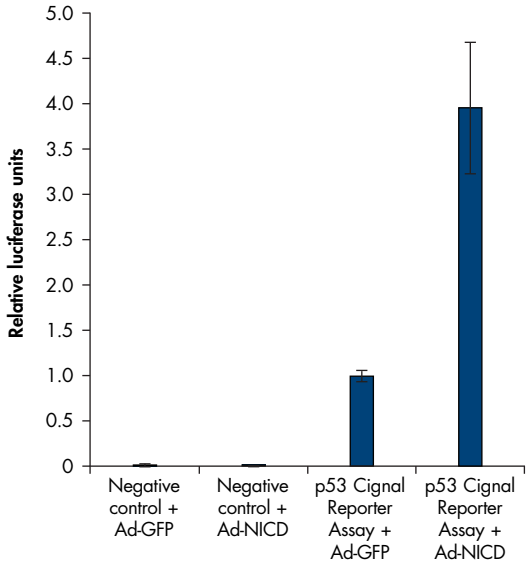

Figure 8. p53 Cignal Reporter Assays show that Notch signaling positively regulates p53 signaling. Transfections were carried out in 293-H cells with p53 Cignal Reporter Assay, negative control, and positive control. After 24 hours of transfection, cells were infected with 100 MOI of recombinant adenovirus expressing constitutively active Notch1 (Ad-NICD) or 100 MOI of recombinant adenovirus expressing GFP (Ad-GFP). A 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

Cell-based reporter assays are commonly used in the study of transcriptional regulation. Cignal Reporter Assays are pathway-focused, cell-based assays that cover a broad range of biological areas, and are an invaluable tool to monitor changes in the activities of signaling pathways. Cignal Reporter Assay Kits are available for pathways crucial in cancer biology (Notch, Wnt/β-Catenin, TGFβ, p53, HIF, Myc, E2F, NFκB), cell cycle control (E2F, p53, Myc), immunology (NFκB, NFAT, Type I IFN, IFNγ signaling), cell proliferation (MAPK/JNK, MAPK/ERK, C/EBP, Myc), developmental biology (Notch, Wnt/β-Catenin, TGFβ), nuclear hormone receptor biology (estrogen receptor, glucocorticoid receptor, retinoic acid receptor), hypoxia signaling (HIF, p53, E2F, Myc), G-coupled protein receptor signaling (MAPK/ERK, NFAT, CRE), PKC/CA^{++} signaling, and cAMP/PKA signaling.

Cignal Reporter Assays 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 speed of measuring changes in transcription. Cignal Reporter Assays provide exceptional versatility as a result of the proprietary design of each TRE, coupled with the broad dynamic range of the luciferase assay system. Researchers can reliably monitor both up- and downregulation of signaling activities using these assays. For example, the NFκB Cignal Reporter Assay was shown to monitor both the upregulation of NFκB signaling by hTNFα protein as well as the downregulation of NFκB signaling by RelA shRNA and NFκB siRNA (Figures 5–6).

Cignal Reporter Assay Kits offer the unique advantage of ready-to-transfect constructs. This provides the reliability and convenience of experiments that can be carried out by direct use of the kit components, eliminating the time-consuming tasks of construct amplification and purification.

The application results detailed in Figures 7 and 8 confirm that Cignal Reporter Assay Kits are outstanding tools for functional genomics studies to assess the biological impact of siRNA, shRNA, and cDNA. Additionally, this technology will accelerate the speed at which researchers elucidate the physiological functions and off-target effects of small chemical molecules and recombinant proteins.

Taken together, these results indicate that Cignal Reporter Assays provide outstanding sensitivity, reproducibility, versatility, and convenience for carrying out quantitative assessments of the regulation of signal transduction pathways.

Ordering Information

Product	Contents	Cat. no.
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Cignal Lenti Reporter Assays	1 or 8 tubes with inducible firefly luciferase or GFP reporter	336851
Cignal Reporter Controls	Positive or negative controls with GFP or luciferase	336881
Cignal Lenti Reporter Controls	Positive or negative controls with GFP, RFP, or luciferase	336891
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HiPerFect Transfection Reagent (0.1 ml)	HiPerFect Transfection Reagent for up to 33 transfections in 24-well plates or up to 133 transfections in 96-well plates	301702

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