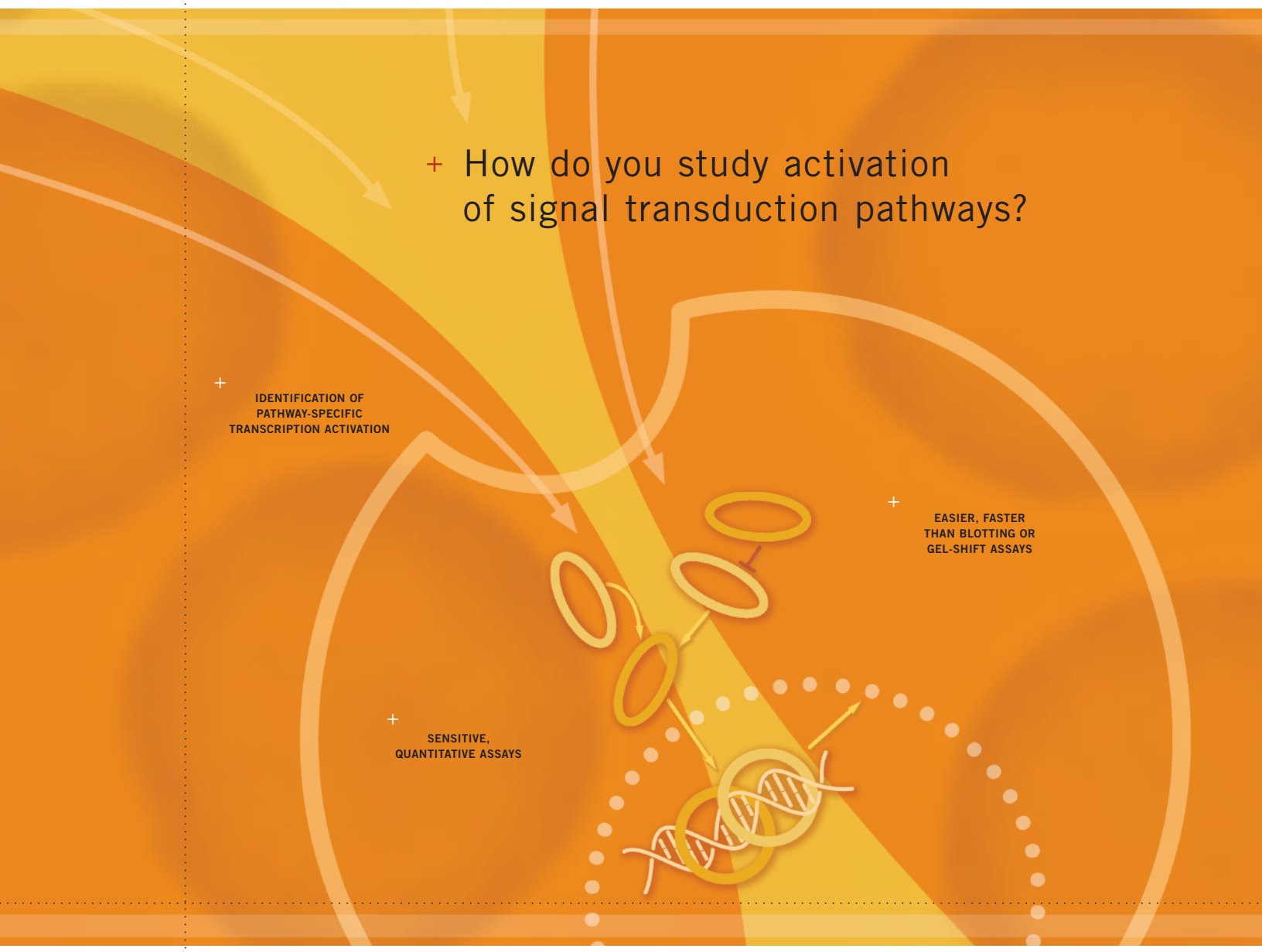
CELL BIOLOGY

Signal Transduction

How do you study activation of signal transduction pathways?



STRATAGENE®

Linking Gene Discovery to Function

As the research focus shifts from gene discovery to functional genomics, scientists need to link genes and proteins to their biological processes. The PathDetect® Pathway Profiling System measures transcription activation quantitatively and with greater sensitivity than cumbersome blotting or gel-shift assays. Previously, tedious blotting procedures were required to monitor transcription of the cellular genes that indicate pathway activation. Alternatively, researchers used time-consuming, non-quantitative gel-shift assays (EMSA) to measure transcription factor-binding activity. With the PathDetect products, reporter enzymes replace cellular genes, and simple enzyme assays provide readout of transcription activation.

CONTENTS:

E2F-Mediated Transcription

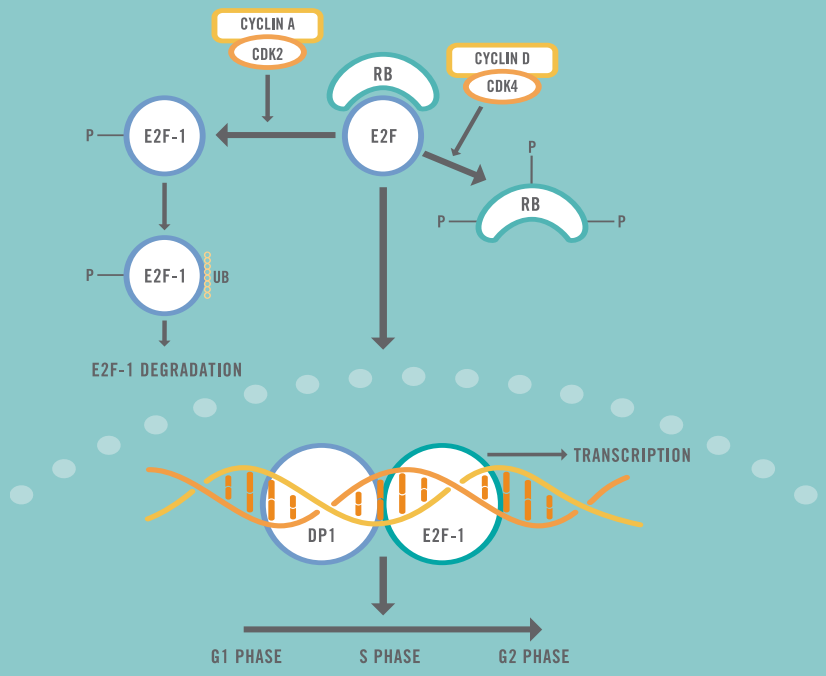


Table 1: The PathDetect® Pathway Profiling System

	RECOMMENDED APPLICATION	AVAILABLE REPORTER SYSTEM
Antibodies Against Transcription Factors	+ Validate expression of transcription factors + Western blotting	+ N/A
PathDetect® *Cis*-Reporting Systems	+ Assessment of *in vivo* activation of pathways	+ GFP, Luciferase
PathDetect® HLR Stable Cell Lines	+ Assessment of effects of extracellular stimuli on pathway activation without transfection	+ Luciferase
PathDetect® *Trans*-Reporting Systems	+ Identification of stimuli that leads to the phosphorylation of a test transcription factor	+ Beta-galactosidase, Luciferase, SEAP

PathDetect® *Cis*-Reporting Systems

Initial Characterization of an Unknown Gene

For many signal transduction pathways, the final step of activation is the binding of an activated transcription factor to an enhancer element. The PathDetect® *Cis*-Reporting Systems determine whether a new gene product or compound activates pathways leading to specific enhancers, using luciferase or hrGFP* expression.

How the PathDetect® *Cis*-Reporting Systems Work

When PathDetect *Cis*-reporting and expression plasmids encoding a gene of interest are co-transfected into mammalian cells, increased reporter activity indicates transcription activation and involvement of the gene product in the pathway leading to the enhancer element (Figures 1, 2). We offer a variety of *Cis*-reporting systems (Table 2) to provide you with simple, rapid, and convenient assessment of *in vivo* activation of pathways, using luciferase reporter or hrGFP expression.

For ultimate flexibility, the pLuc-MCS vector includes a multiple cloning site situated for easy insertion of the enhancer or transcription element of your choice. The system also includes positive and negative controls that ensure the success of your experiments.

For more information about our PathDetect systems, please visit www.stratagene.com/transcription.

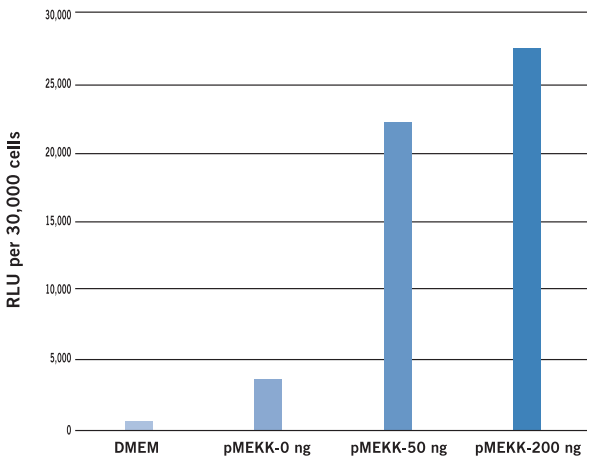


Figure 1
DETECTION OF TRANSCRIPTION ACTIVATION BY LUCIFERASE ACTIVITY.
1 µg of the pNF-κB-Luc plasmid was cotransfected into HeLa cells with increasing amounts of the pFC-MEKK plasmid, serving as the positive control. Luciferase activity was measured 48 hours after transfection. The results show virtually no luciferase activity in untransfected HeLa cells, background levels of transcription in cells carrying only the pNF-κB-Luc plasmid, and several-fold amplification of signal from HeLa cells expressing MEKK.

AP-1	Activator protein-1
c/EBP	CCAAT-enhancer binding protein
CRE	Cyclic AMP
DR1/RXRE	Retinoic x receptor epsilon
DR3/VDR	Vitamin D receptor
DR4/TR	Thyroid receptor
DR5/RARE	Retinoic acid receptor epsilon
Egr-1	Early growth response-1
GAS	γ-activated sequence
GRE	Glucocorticoid response element
ISRE	Interferon-stimulated response element
LILRE	LPS IL-1β response element
NFAT	Nuclear factor of activated T cells
NF-κB	Nuclear factor κB
p53	Tumor suppressor protein p53
SRE	Serum response element
SRF	Serum response factor
TARE	TGF-Beta or Activin response element

Table 2
PATHDETECT® *CIS*-REPORTING SYSTEMS AVAILABLE FROM STRATAGENE

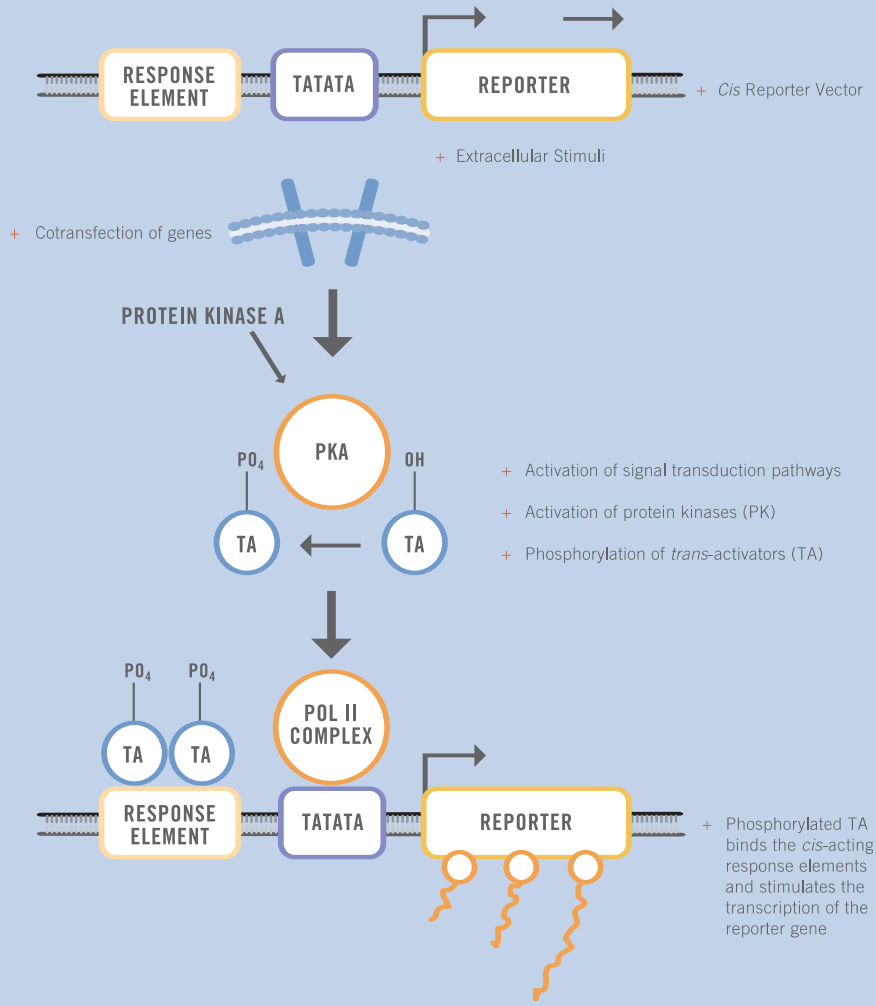


Figure 2
HOW THE PATHDETECT® *CIS*-REPORTING SYSTEMS WORK
Extracellular signals trigger the activation of a series of intracellular signaling molecules such as protein kinases and phosphatases. Many of these signal transduction pathways converge at transcription factors that bind to specific enhancer elements found in the promoters of various genes and modulate the transcription of these genes. The activation of a signal transduction pathway can, therefore, be monitored by the expression level of the luciferase reporter gene controlled by a promoter containing these enhancer elements.

PathDetect® *Trans*-Reporting Systems

Identify Stimuli Leading to Phosphorylation of a Transcription Factor

While the *cis*-reporting systems are useful for initial characterization of an unknown gene product, the PathDetect *trans*-reporting systems** are ideal for determining the direct or indirect involvement of new gene products, growth factors, and drug candidates on specific pathways.

How the PathDetect® *Trans*-Reporting Systems Work

When the pathway-specific fusion *trans*-activator and reporter plasmids are co-transfected with the contract expressing your gene of interest into mammalian cells, increased reporter activity indicates that your gene product of interest directly or indirectly phosphorylates the activation domain of the fusion *trans*-activator protein. (Figures 3, 4).

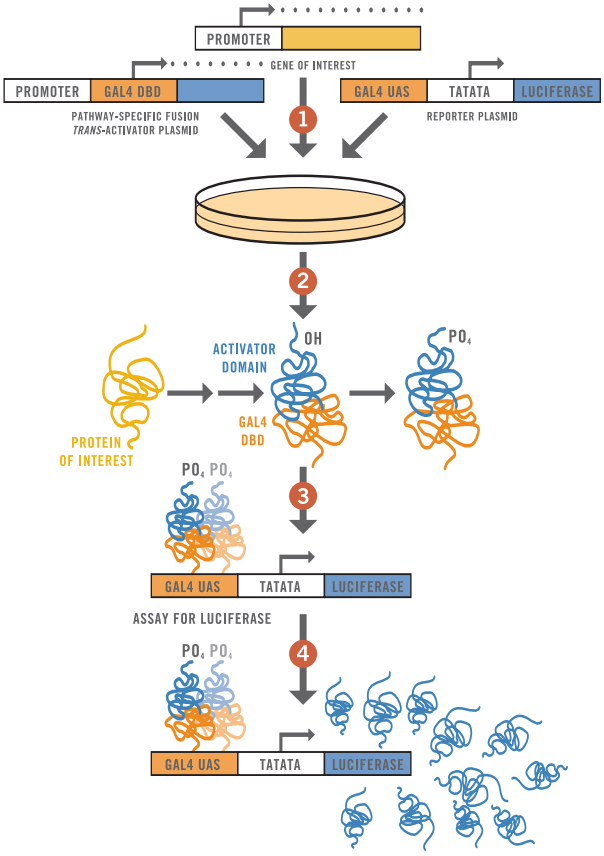
We offer a variety of trans-reporting systems (Table 3) as plasmid vectors with luciferase reporter protein for cotransfection into the cell line of your choice or as stable HeLa luciferase reporter (HLR) cell lines*** for direct assessment of extracellular stimuli without transfection. Luciferase is the reporter protein of choice for the PathDetect system due to its high level of sensitivity and simple protocol. However, alternate reporter plasmids utilizing β-galactosidase or secreted alkaline phosphatase (SEAP) as reporter proteins are available.

For ultimate flexibility, the pFA-CMV vector includes a multiple cloning site for inserting and expressing any transcription factor of interest. The system also includes positive and negative controls that ensure the success of your experiments.

For more information about our PathDetect systems, please visit www.stratagene.com/transcription.

Table 3: PathDetect Trans-Reporting Systems Available from Stratagene

ATF-2	MAPK (mitogen-activated protein kinase) pathway
c-Fos	JNK (c-Jun N-terminal kinase) pathway
c-Jun	JNK (c-Jun N-terminal kinase) pathway
CHOP/GADD153	p38 Kinase pathway
CREB	PKA (cAMP-dependent kinase) pathway
Elk1	MAPK (mitogen-activated protein kinase) pathway



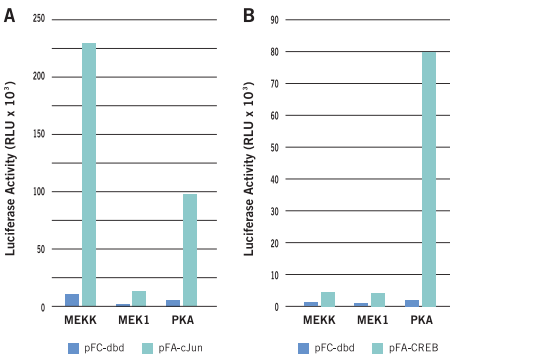


Figure 3
DETECTION OF TRANSCRIPTION ACTIVATION BY LUCIFERASE ACTIVITY.
The pFR-Luc reporter plasmid (1 µg) was cotransfected with 10 ng fusion *trans*-activator plasmid plus 50 ng of the indicated control vector using lipofection methods. After transfection, cells were lysed and assayed for luciferase expression. The pFC-dbd plasmid expresses only the GAL4 DNA binding domain and is used as a negative control.

Figure 4
HOW THE PATHDETECT TRANS-REPORTING SYSTEMS WORK
1. The construct expressing your gene of interest, the pathway-specific fusion *trans*-activator plasmid and the reporter plasmid are cotransfected into mammalian cells. **2.** The fusion *trans*-activator plasmid expresses the chimeric fusion *trans*-activator protein which is the GAL4 DNA binding domain (dbd) fused to the specific activation domain. Your gene product of interest directly or indirectly phosphorylates the activation domain of the fusion transactivator protein. **3.** The phosphorylated fusion *trans*-activator protein binds to the GAL4 UAS of the reporter which activates luciferase expression. **4.** Luciferase expression is detected using a simple assay (Figure 3).

Antibodies against Transcription Factors

For the detection of transcription factors, we offer a line of highly specific polyclonal antibodies, optimized for Western blotting applications.

Table 4: Antibodies Available from Stratagene

PRODUCT NAME	SIZE	CAT. NO.
Anti-Akt3/PKB gamma	100 µg	B10000
Anti-Annexin II	100 µg	B10001
Anti-BMP-2	100 µg	B10002
Anti-BMP-3	100 µg	B10003
Anti-Caspase 3	100 µg	B10004
Anti-Catenin beta	100 µg	B10005
Anti-Cdc25c	100 µg	B10006
Anti-Cdc27	100 µg	B10048
Anti-Cdk2	100 µg	B10007
Anti-Cdk4	100 µg	B10008
Anti-Cdk5	100 µg	B10009
Anti-Cdk6	100 µg	B10010
Anti-Cdk9	100 µg	B10011
Anti-c-Myc	100 µg	B10012
Anti-Cyclin F	100 µg	B10013
Anti-Cyclin H	100 µg	B10014
Anti-D4-GDI	100 µg	B10015
Anti-DR4/TRAIL-R1 NT	100 µg	B10016
Anti-E2F1	100 µg	B10017
Anti-EGFR	100 µg	B10018
Anti-ERK 2	100 µg	B10019
Anti-ERK 6	100 µg	B10020
Anti-FGF-2	100 µg	B10021
Anti-FGF-9	100 µg	B10022
Anti-FLIP (CT)	100 µg	B10023
Anti-GFR alpha-1/RETL1/TrnR1	100 µg	B10024
Anti-Histone H3	100 µg	B10025
Anti-HSP40	100 µg	B10026
Anti-HSP47	100 µg	B10027
Anti-HSP60	100 µg	B10028
Anti-IGF-I	100 µg	B10029
Anti-INK4c (p18)	100 µg	B10030
Anti-Jun (Ser63)	100 µg	B10031
Anti-Jun (Ser73)	100 µg	B10032

PRODUCT NAME	SIZE	CAT. NO.
Anti-Kip2 p57	100 µg	B10033
Anti-MC1-R	100 µg	B10034
Anti-MC4-R	100 µg	B10035
Anti-MC5-R	100 µg	B10036
Anti-Neu	100 µg	B10037
Anti-N-Myc 1	100 µg	B10038
Anti-PCNA	100 µg	B10039
Anti-PP2A C-subunit	100 µg	B10040
Anti-Rad9	100 µg	B10041
Anti-RIP Kinase	100 µg	B10042
Anti-Rsk 2/MAPKAP Kinase 1b	100 µg	B10043
Anti-SKK2/MEK3	100 µg	B10044
Anti-Tak1 (CT)	100 µg	B10045
Anti-TRADD	100 µg	B10046
Anti-TRAF-4	100 µg	B10047

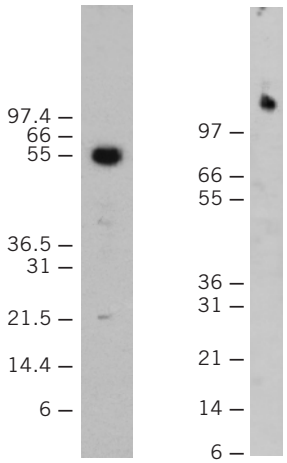


Figure 5

(Left) Western blot analysis of FLIP (c-terminus) using HeLa cell lysate. A band of approximate molecular weight of 55 kD was detected.

(Right) Western blot analysis of EGFR using HeLa cell lysate. A band of approximate molecular weight of 134 kD was detected.

Related Products

GeneJammer® Transfection Reagent

Our GeneJammer reagent combines high transfection efficiency with low cytotoxicity, all in a simple protocol. Simply mix the GeneJammer reagent with your DNA, add it to your cells and assay in 48 hours.

For more information about our transfection reagents, please visit us at www.stratagene.com.

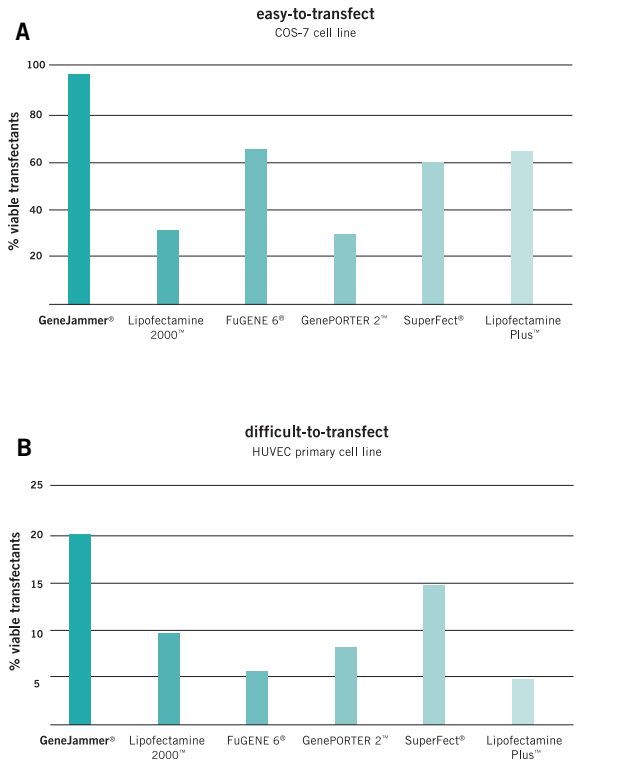


Figure 6
TRANSFECTION USING GENEJAMMER® REAGENT IN EASY- AND DIFFICULT-TO-TRANSFECT CELLS.
The pCMV-β-gal reporter plasmid was transfected into COS-7 (Panel A) and human umbilical vein endothelial cells (HUVEC, Panel B). Transfection conditions were optimized for GeneJammer reagent and each of the five most common transfection reagents.

Ordering Information

PRODUCT NAME	DESCRIPTION	SIZE	CONTENTS	CATALOG NO.
PathDetect® *Cis*-Reporter Systems, Plasmids Using Luciferase Reporter				
AP-1 (ACTIVATOR PROTEIN-1)	*Cis*-Reporting System	50 assays	pAP-1-Luc plasmid, pFC-MEKK control plasmid	219073
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	219074
C/EBP (CCAAT-ENHANCER BINDING PROTEIN)	*Cis*-Reporting System	50 assays	pc/EBP-Luc plasmid, pCIS-CK control plasmid	240111
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240112
CRE (CYCLIC AMP)	*Cis*-Reporting System	50 assays	pCRE-Luc plasmid, pFC-PKA control plasmid	219075
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	219076
DR1 /RXRE (RETINOIC X RECEPTOR EPSILON)	*Cis*-Reporting System	50 assays	pDR1-Luc plasmid, pCIS-CK control plasmid	240113
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240114
DR3/VDR (VITAMIN D RECEPTOR)	*Cis*-Reporting System	50 assays	pDR3-Luc plasmid, pCIS-CK control plasmid	240115
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240116
DR4/TR (THYROID RECEPTOR)	*Cis*-Reporting System	50 assays	pDR4-Luc plasmid, p*CIS*-CK control plasmid	240135
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240136
DR5/RARE (RETINOIC ACID RECEPTOR EPSILON)	*Cis*-Reporting System	50 assays	pDR5-Luc plasmid, pCIS-CK control plasmid	240119
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240120
EGR-1 (EARLY GROWTH RESPONSE-1 FACTOR)	*Cis*-Reporting System	50 assays	pEgr-1-Luc plasmid, pCIS-CK control plasmid	240129
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240130
GAS (γ-ACTIVATED SEQUENCE)	*Cis*-Reporting System	50 assays	pGAS-Luc plasmid, pCIS-CK control plasmid	219093
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	219091
GRE (GLUCOCORTICOID RESPONSE ELEMENT)	*Cis*-Reporting System	50 assays	pGRE-Luc plasmid, pCIS-CK control plasmid	240133
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240134
ISRE (INTERFERON-STIMULATED RESPONSE ELEMENT)	*Cis*-Reporting System	50 assays	pISRE-Luc plasmid, pCIS-CK control plasmid	219092
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	219089
LILRE (LPS IL-1B RESPONSE ELEMENT)	*Cis*-Reporting System	50 assays	pLILRE-Luc plasmid, pCIS-CK control plasmid	240131
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240132
NFAT (NUCLEAR FACTOR OF ACTIVATED T CELLS)	*Cis*-Reporting System	50 assays	pNFAT-Luc plasmid, pCIS-CK control plasmid	219094
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	219088
NF-κB (NUCLEAR FACTOR κ B)	*Cis*-Reporting System	50 assays	pNF-κB-Luc plasmid, pFC-MEKK control plasmid	219077
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	219078
P53 (TUMOR SUPPRESSOR PROTEIN P53)	*Cis*-Reporting System	50 assays	p53-Luc plasmid, pFC-p53 control plasmid	219083
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	219085
SRE (SERUM RESPONSE ELEMENT)	*Cis*-Reporting System	50 assays	pSRE-Luc plasmid, pFC-MEKK control plasmid	219079
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	219080
SRF (SERUM RESPONSE FACTOR)	*Cis*-Reporting System	50 assays	pSRF-Luc plasmid, pFC-PKA control plasmid	219081
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	219082
TARE (TGF-BETA OR ACTIVIN RESPONSE ELEMENT)	*Cis*-Reporting System	50 assays	pTARE-Luc plasmid, pCIS-CK control plasmid	219095
	Cis-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240039
PathDetect® *Cis*-Reporter Plasmids Using hrGFP Reporter Protein				
pAP-1-hrGFP PLASMID	*Cis*-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240049
pCRE-hrGFP PLASMID	*Cis*-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240050
pNFAT-hrGFP PLASMID	*Cis*-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240053
pNF-κB-hrGFP PLASMID	*Cis*-Reporting Plasmid	50 µg	*Cis*-reporter plasmid	240051
PathDetect® *Cis*-Reporter Multiple Cloning Site Vector				
pLUC-MCS PLASMID	Multiple Cloning Site Vector	50 µg	*Cis*-reporter cloning vector	219087

PRODUCT NAME	DESCRIPTION	SIZE	CATALOG NO.
PathDetect® *Cis*-Reporter Control Plasmids			
pCIS-CK PLASMID	Negative control plasmid Recommended for use with all *Cis*-reporter plasmids	50 µg	219090
pFC-MEKK PLASMID	Positive control plasmid Recommended for use with pNF-κB-hrGFP, pNF-κB-Luc, pAP-1-Luc, pAP-1-hrGFP, and pSRE-Luc plasmids	10 µg	219059
pFC-P53 PLASMID	Positive control plasmid Recommended for use with p53-Luc plasmid	10 µg	219084
pFC-PKA PLASMID	Positive control plasmid Recommended for use with pCRE-hrGFP, pCRE-Luc, and pSRF-Luc plasmids	10 µg	219071

Ordering Information

PRODUCT NAME	DESCRIPTION	SIZE	CONTENTS	CATALOG NO.
PathDetect® *Trans*-Reporting Systems				
C-JUN (JNK (C-JUN N-TERMINAL KINASE) PATHWAY)	*Trans*-Reporting System	50 transfection assays	pFR-Luc, pFA2-cJun, pFC2-dbd and pFC-MEKK plasmids	219000
CHOP (P38 KINASE PATHWAY)	*Trans*-Reporting System	50 transfection assays	pFR-Luc, pFA-CHOP, pFC2-dbd and pFC-MEK3 plasmids	219015
CREB (PKA (CAMP-DEPENDENT KINASE) PATHWAY)	*Trans*-Reporting System	50 transfection assays	pFR-Luc, pFA2-CREB, pFC2-dbd and pFC-PKA plasmids	219010
ELK1 (MAPK (MITOGEN-ACTIVATED PROTEIN KINASE) PATHWAY)	*Trans*-Reporting System	50 transfection assays	pFR-Luc, pFA2-Elk1, pFC2-dbd and pFC-MEK1 plasmids	219005

PRODUCT NAME	DESCRIPTION	SIZE	CONTENTS	CATALOG NO.
PathDetect® *Trans*-Reporter Cell Lines				
HLR CELL LINE	Stable HeLa luciferase reporter cell line	1 x 10^6 cells	pFA2-CREB, pFC-PKA, and pFC-CMV control plasmid	800050
HLR-CHOP CELL LINES	Stable HeLa luciferase reporter cell line	1 x 10^6 cells	pFC-CMV control plasmid, pFC-MEK3 control plasmid	800060
HLR-CREB CELL LINE	Stable HeLa luciferase reporter cell line	1 x 10^6 cells	pFC-PKA control plasmid, pFC-CMV control plasmid	800065
HLR-ELK1 CELL LINE	Stable HeLa luciferase reporter cell line	1 x 10^6 cells	pFC-MEK1 control plasmid, pFC-CMV control plasmid	800055

PRODUCT NAME	DESCRIPTION	SIZE	CATALOG NO.
PathDetect® *Trans*-Reporter Plasmids			
pFR-β-GAL PLASMID	β-galactosidase reporter plasmid	50 µg	219002
pFR-CAT PLASMID	Chloroamphenicol acetyltransferase reporter plasmid	50 µg	219001
pFR-LUC PLASMID	Luciferase reporter plasmid included in all PathDetect systems	50 µg	219050
pFR-SEAP PLASMID	Secreted alkaline phosphatase reporter plasmid	50 µg	219004
PathDetect® *Trans*-Reporter Activator Plasmids			
pFA-ATF2 PLASMID	Fusion *Trans*-activator plasmid	10 µg	219026
pFA-CFOS PLASMID	Fusion *Trans*-activator plasmid	10 µg	219031
pFA2-CJUN PLASMID	Activator plasmid for c-Jun reporting system	10 µg	219053
pFA2-CREB PLASMID	Activator plasmid for CREB reporting system	10 µg	219068
pFA-CHOP PLASMID	Fusion *Trans*-activator plasmid for CHOP reporting system	10 µg	219054
pFA2-ELK1 PLASMID	Activator plasmid for Elk1 reporting system	10 µg	219062
PathDetect® *Trans*-Reporter Cloning Vector			
pFA-CMV PLASMID	Fusion *Trans*-activator cloning vector	20 µg	219036
PathDetect® *Trans*-Reporter Control Plasmids			
pFC2-DBD PLASMID	Negative control plasmid for all PathDetect *Trans*-systems	10 µg	219056
pFC-MEK1 PLASMID	Positive control plasmid for Elk1 reporting system	10 µg	219065
pFC-MEK3 PLASMID	Positive control plasmid	10 µg	219086
pFC-MEKK PLASMID	Positive control plasmid for c-Jun reporting system	10 µg	219059
pFC-PKA PLASMID	Positive control plasmid for CREB reporting system	10 µg	219071
Related Products			
GENEJAMMER® TRANSFECTION REAGENT	Polyamine transfection reagent	0.3 ml	204132
		1.0 ml	204130
		4 x 1.0 ml	204131
LUCIFERASE ASSAY KIT	Rapid and sensitive detection of luciferase activity	100 reactions	219020

LEGAL

* Patents pending. Certain hrGFP products, and other products containing hrGFP variants, require a license from Stratagene. For license information, please contact Business Development, 858-535-5400 (phone) or 858-535-0071 (fax).

**Use of the CMV promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation and licensed FOR RESEARCH USE ONLY.

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