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Articles Citing Promega Products in Novel and Interesting Applications

...current citations adapted from peer-review journal articles featuring Promega products in interesting or novel applications. We welcome suggestions for similar citations of general interest to our readers.

TGF-beta signaling

Targeted deletion of *Smad4* shows it is required for transforming growth factor beta and activin signaling in colorectal cancer cells. Zhou, S.[1,2], Buckhaults, P.[2], Zawel, L.[2], Bunz, F.[1,2], Riggins, G.[2], Le Dai, J.[2,3], Kern, S.E.[2,3], Kinzler, K.W.[2] and Vogelstein, B.[1,2] (1998) *Proc. Natl. Acad. Sci. USA* **95**, 2412.

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In this report, the hypothesis that *Smad4* might be required for transmitting signals from the transforming growth factor (TGF) beta family was tested directly by deleting the gene using homologous recombination. *Smad4* was deleted in human colorectal cancer cells resulting in the abrogation of signaling through TGFbeta and activin, a TGFbeta family member. This finding helps in understanding the role *Smad4* plays in tumorigenesis. Targeted deletion of both alleles for *Smad4* was performed, and clonal lines were transfected with an expression plasmid containing TGFbeta type II receptor plus alpha-1,3-galactosyltransferase in the presence or absence of TGFbeta. Promega's **Luciferase Assay Reagent**[(a)] was used to quantitate reporter activity in cell lysates.

Ordering Information

Product	Size	Cat.#
Luciferase Assay Reagent	1,000 assays	E1483
Luciferase Assay System[(a)]	100 assays	E1500
Luciferase Assay System with Reporter Lysis Buffer[(a)]	100 assays	E4030

DNA subcloning/amplification

Modulation of the chaperone heat shock cognate 70 by embryonic (pro)insulin correlates with prevention of apoptosis. de la Rosa, E.J., Vega-Núñez, E., Morales, A.V.[1], Serna, J., Rubio, E. and de Pablo, F. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 9950.

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In developing chicken embryos, prepancreatic proinsulin prevents apoptosis during neurulation. In efforts to characterize the survival effects of insulin, these researchers discovered the insulin-dependent regulation of the molecular chaperone heat shock cognate 70kDa (Hsc70). Levels of Hsc70 were found to be dependent upon endogenous levels of proinsulin. In addition, apoptosis was seen in cells with the lowest levels of Hsc70. The result suggests that embryonic proinsulin modulates the role of Hsc70 in preventing apoptosis during early development. It also demonstrates a role for a molecular chaperone in embryogenesis. The **pGEM®-T Vector System**[(b,c)] was used for subcloning and sequencing the Hsc70 RT-PCR product. The fragment was labeled with digoxigenin-dUTP and used to screen a chicken embryo (E1.5) cDNA library. Phage from positive plaques were purified using the **Wizard® Lambda Preps DNA Purification System**[(d)].

Ordering Information

Product	Size	Cat.#
pGEM®-T Vector System I	20 reactions	A3600
pGEM®-T Vector System II	20 reactions	A3610
Wizard® Lambda Preps DNA Purification System	20 preps	A7290

The pGEM®-T Vector System II is supplied with High Efficiency JM109 Competent Cells.

in vitro transcription/translation

Molecular cloning and characterization of human caspase-activated DNase. Mukae, N.[1], Enari, M.[1], Sakahira, H.[1], Fukuda, Y.[2], Inazawa, J.[2], Toh, H.[3] and Nagata, S.[1] (1998) *Proc. Natl. Acad. Sci. USA* **95**, 9123.

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Shigekazu Nagata and colleagues isolated a caspase-3-activated DNase, CAD, which is specifically activated during programmed cell death (PCD) and is responsible for cleaving the chromosomal DNA of dying cells. CAD normally exists as a complex with its inhibitor, ICAD, and cleavage of ICAD by caspase-3 allows release of active CAD.

In this report, two classes of human CAD cDNAs were isolated. Although one appeared to be the product of a pseudogene, the other formed a functional CAD protein (hCAD) when transcribed and translated in vitro in the presence of ICAD. Results of the expression and overexpression of the CAD protein in human cell lines correlated with DNA fragmentation during PCD. The **TNT® Coupled Reticulocyte Lysate System**[(a,e,f)] was used to produce functional hCAD from the human cDNA clone.

Ordering Information

Product	Size	Cat.#
TNT® T7 Coupled Reticulocyte Lysate System	40 reactions	L4610

in vitro transcription/translation

Essential role of CED-4 oligomerization in CED-3 activation and apoptosis. Yang, X.[1,3], Chang, H.Y.[1] and Baltimore, D.[1,2] (1998) *Science* **281**, 1355.

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Aberrant apoptotic mechanisms that prevent normal cell death are suspected in many cancers. This report concentrated on understanding the intracacies of apoptosis with the goal of offering some protective measure against cancer. The focus was on how the interaction of three core components, CED-3, CED-4 and CED-9, of the death cascade in *C. elegans* regulate cell death. Fusion of the CED-3 protease domain with three copies of FK506-binding protein (Fkp-CED-3) was performed to test conversion of CED-3 to an active enzyme, similar to other caspase zymogens. Oligomerization of the construct, using a dimeric ligand for Fkp, resulted in a dose-dependent and saturable increase in CED-3 cleavage and apoptotic activity. Fkp-CED-3 was produced by in vitro coupled transcription/translation using the **TNT® T7 Coupled Reticulocyte Lysate System**[(a,e,f)].

Ordering Information

Product	Size	Cat.#
TNT® T7 Coupled Reticulocyte Lysate System	40 reactions	L4610

in vitro transcription/translation

Expression of the catalytic subunit (UL54) and the accessory protein (UL44) of human cytomegalovirus DNA polymerase in a coupled in vitro transcription/translation system. Cihlar, T., Fuller, M.D. and Cherrington, J.M. (1997) *Protein Expr. Purif.* **11**, 209.

Gilead Sciences, 333 Lakeside Drive, Foster City, California 94404 USA

In this paper, expression and function of an in vitro expressed DNA polymerase 140kDa subunit and 58kDa accessory protein from human cytomegalovirus (HCMV) were investigated. The HCMV proteins were expressed from supercoiled **pGEM®-3Zf() Vector**[(b)] in the **TNT® SP6 Coupled Reticulocyte Lysate System**[(b,c,d)]. To make the expression constructs, the genes encoding the catalytic subunit (UL54) and the accessory protein (UL44) of human cytomegalovirus (HCMV) were cloned into the pGEM®-3Zf() Vector in the orientation corresponding with the SP6 promoter sense strand. The influence of various 5´-untranslated regions (5´-UTRs) on expression efficiency was also investigated, and constructs were made by intramolecular ligation of 5´-UTR regions from alfalfa mosaic virus (AMV) RNA4 and HCMV. Circular plasmids were purified using the **Wizard® *Plus* Minipreps DNA Purification System**[(d)], ethanol-precipitated and expressed in the TNT® System.

A truncated form of the 5´-UTR from AMV substantially increased expression of both the UL54 and UL44 proteins. The in vitro expressed 140kDa catalytic subunit (UL54) was compared to the native HCMV enzyme for DNA polymerase and 3´->5´ exonuclease activities. These activities were found to be dependent on salt concentration in both in vitro expressed and native proteins. The in vitro expressed UL54 was similar to the native enzyme in affinity for nucleotides and sensitivity to inhibitors. These results demonstrate the robust performance of the TNT® Systems for producing high-molecular weight yet functional proteins in vitro.

Ordering Information

Product	Size	Cat.#
TNT® SP6 Coupled Reticulocyte Lysate System	40 reactions	L4600
pGEM®-3Zf() Vector	20µg	P2261
Wizard® *Plus* Minipreps DNA Purification Systems	50 preps	A7100
	100 preps	A7500
	150 preps	A7510

kinase signaling

NF-kappaB-inducing kinase activates IKK-alpha by phosphorylation of Ser-176. Ling., Cao, Z. and Goeddel, D.V. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 3792.

Tularik, Inc., South San Francisco, CA 94080 USA

NF-kappaB activation is controlled by two successively acting kinases, NF-kappaB-inducing kinase (NIK) and two IkappaB kinases (IKK-alpha and IKK-beta). In this report, NIK was shown to preferentially phosphorylate IKK-alpha over IKK-beta, on Ser^{176} in the enzyme's activation loop. Two mutants of IKK-alpha containing alanine and glutamic acid at position 176, respectively, led to either a form of the kinase that could not be phosphorylated or a form that was constitutively active. The former, a nonphosphorylatable Ala^{176} mutant, acts as a dominant negative inhibitor of interleukin 1- and tumor necrosis factor-induced NF-kappaB activation. Apparently phosphorylation of Ser^{176} by NIK is necessary for cytokine-mediated NF-kappaB activation. For these studies, wildtype and mutant sequences of IKK-alpha were subcloned into expression vectors and cotransfected with a luciferase reporter vector into 293, 293/IL-1RI and HeLa cells. The **Luciferase Assay System**[(a)] was used to determine reporter gene activity.

Ordering Information

Product	Size	Cat.#
Luciferase Assay System	100 assays	E1500

Luciferase Assay System with Reporter Lysis Buffer	100 assays	E4030
Luciferase Assay Reagent[a]	1,000 assays	E1483

luciferase activity

Noncytopathic Sindbis virus RNA vectors for heterologous gene expression. Agapov., E. V., Frolov, I., Lindenbach, B.D., Pragai, B.M., Schlesinger, S. and Rice, C.M. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 12989.

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Recombinant Sindbis viruses (SIN) and replicons are capable of high expression levels of foreign genes in vertebrate cells, but their utility as vectors has been limited by cytopathic effects from replication of the viruses. This article describes the application of Sindbis vectors that have been selected for their ability to grow noncytopathically in BHK cells and produce long-term expression of foreign genes. A noncytopathic replicon (S1) was used to develop vectors for long-term expression of heterologous sequences, including reporter genes such as *lac*Z (encoding beta-galactosidase) and *luc* (encoding *Coleoptera* luciferase). Promega's **Luciferase Assay System**[a] was used to measure luciferase expression from a defective-interfering SIN construct (DI/*luc*), complemented in *trans* by a replicon (SINrep18). Uninduced cells had a low, constitutive level of luciferase activity that was proportional to the amount of virus added. When these cells were transfected with SINrep18 and selected, abundant luciferase expression accumulated in 5 days and was maintained for at least 4 additional days, demonstrating that these noncytopathic replicons are capable of prolonged expression leading to accumulation of high protein levels.

Ordering Information

Product	**Size**	**Cat.#**
Luciferase Assay System	100 assays	E1500
Luciferase Assay System with Reporter Lysis Buffer	100 assays	E4030
Luciferase Assay Reagent[a]	1,000 assays	E1483

luciferase activity, protein kinase activity, coupled transcription/translation

Activation of androgen receptor function by a novel nuclear protein kinase. Moilanen, A.-M.[1], Karvonen, U.[1], Poukka, H.[1], Jänne, O.A.[1,2] and Palvimo, J.J.[1] (1998) *Mol. Biol. Cell* **9**, 2527.

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Moilanen and colleagues report the identification of a protein, the androgen receptor-interacting nuclear protein kinase (ANPK), which is capable of interacting with the zinc finger region of the androgen receptor (AR). The catalytic domain of ANPK shares extensive homology with other protein kinases, including one that may contribute to Down Syndrome learning defects. ANPK interacts with AR in a hormone-dependent manner, is able to mediate AR-mediated transcription and is a functionally active protein kinase.

In transient transfection assays ANPK overexpression enhanced AR-dependent transcription of a luciferase reporter gene, driven by the AR-regulated probasin promoter, as determined using Promega's **Luciferase Assay Reagent**[a]. ANPK cDNA constructs were expressed in the **TNT® T7 Coupled Reticulocyte Lysate System**[a,e,f] and in various cell lines. ANPK was widely expressed and comigrated with a ~160kDa protein in all cell lines and in the TNT® Lysate. The protein kinase activity of ANPK was verified using myelin binding protein, histone H3 and c-Jun as model substrates. However, ANPK was unable to modify other core histones, histone H1 or the nonhistone protein HMG14. Taken together, the data indicate that ANPK substrate specificity differs from those of the cyclin-dependent kinases and MAP kinases. The phosphorylation of AR by ERK2 and **human recombinant cdc2 Kinase** was shown in vitro. However, AR was not phosphorylated by ANPK in vitro, and overexpression of ANPK did not increase AR phosphorylation in vivo. These results indicate that ANPK activation of AR may occur through modification of AR-associated proteins or stabilization of the AR protein, but not direct phosphorylation of the androgen receptor.

Ordering Information

Product	**Size**	**Cat.#**
Luciferase Assay System[a]	100 assays	E1500
Luciferase Assay System with Reporter Lysis Buffer	100 assays	E4030
Luciferase Assay Reagent	1,000 assays	E1483
TNT® T7 Coupled Reticulocyte Lysate System	40 reactions	L4610
rhcdc2 Kinase	100u	V2891

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[a]U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289, and Australian Pat. No. 649289, have been issued to Promega Corporation for a firefly luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay.

[b]U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.

[c]Licensed under one or both of U.S. Pat. No. 5,487,993 and European Pat. No. 0 550 693.

[d]U.S. Pat. Nos. 5,658,548 and 5,808,041 and Australian Pat. No. 689815 have been issued to Promega Corporation for nucleic acid purification on silica gel and glass mixtures.

[e]U.S. Pat. Nos. 5,324,637, 5,492,817 and 5,665,563, European Pat. No. 0 566 714 B1 and Australian Pat. No. 660329 have been issued to Promega Corporation for coupled transcription/translation systems that use RNA polymerases and eukaryotic lysates.

[f]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

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