

References Using Promega Transfection Reagents

Tfx™ Reagents for the Transfection of Eukaryotic Cells

1. Fearon, I.M., *et al.* (1997) Hypoxia inhibits the recombinant α_{1C} subunit of the human cardiac L-type Ca^{2+} channel. *J. Physiol.* **500.3**, 551-556.
Systems Used: Tfx™-50 Reagent.
Summary: The reagent was used to transfect 293 Cells.
2. Gobin, S.J.P. *et al.* (1997) Site α is crucial for two routes of IFN γ -induced MHC Class I transactivation: The ISRE-mediated route and a novel pathway involving CIITA. *Immunity* **6**, 601.
Systems Used: Tfx™-50 Reagent; pGL3 Basic Vector; pGL3 Enhancer Vector; Universal RiboClone® cDNA Synthesis System
Summary: The Tfx™-50 Reagent was used to transfect K562 cells. Cells were assayed for expression of a surface protein by FACS analysis.
3. Gründemann, D. *et al.* (1997) Primary structure and functional expression of the apical organic cation transporter from kidney epithelial LLC-PK1 cells. *J. Biol. Chem.* **272**, 10408.
System Used: Tfx™-50 Reagent.
Summary: The reagent was used to produce transiently transfected LLC-PK1 and 293 cells as well as stably transfected 293 cells.
4. Ichijo, H. *et al.* (1997) Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* **275**, 90.
Systems Used: Tfx™-50 Reagent; Transfectam® Reagent.
Summary: Tfx™-50 Reagent was used to produce transient transfectants of human 293 cells. Eight hours post-transfection, the cells were treated with apoptotic agents for 16 hours and assayed.
5. Inui, T. *et al.* (1997) Cathepsin K antisense oligodeoxynucleotide inhibits osteoclastic bone resorption. *J. Biol. Chem.* **272**, 8109.
System Used: Tfx™-50 Reagent.
Summary: The reagent was used to transiently transfect osteoclasts with antisense oligonucleotides.
6. Keogh, M.-C. *et al.* (1997) High efficiency reporter gene transfection of vascular tissue *in vitro* and *in vivo* using a cationic lipid-DNA complex. *Gene Therapy*, **4**, 162.
System Used: Tfx™-50 Reagent.
Summary: The reagent was used to transfect pGL3 Vector into HepG2, HUVEC, and human, rat and rabbit primary cells. The optimized conditions were used to transfect rabbit arteries *in vivo* and human arteries *in vitro*.
7. Ko, B.C.B. *et al.* (1997) Identification and characterization of multiple osmotic response sequences in the human aldose reductase gene. *J. Biol. Chem.* **272**, 16431.
Systems Used: Tfx™-50; pGL3 Basic Vector; pGL3 Promoter Vector.
Summary: Studies were performed in Chang liver cells using 2 μ g of luciferase reporter and 1 μ g of β -galactosidase control in serum-free medium. Cells were transfected 1hr at 37°C, the medium removed and fresh medium applied. Twenty-four hours later the cells were assayed.
8. Nishitoh, H. *et al.* (1996) Identification of type I and type II serine/threonine kinase receptors for growth/differentiation factor-5. *J. Biol. Chem.* **271**, 21345.
System Used: Tfx™-50 Reagent.
Summary: The reagent was used to transiently transfect COS-1 cells and R mutant Mv1Lu cells.
9. Nork, T.M. *et al.* (1997) p53 regulates apoptosis in human retinoblastoma. *Arch. Ophthalmol.* **115**, 213.
System Used: Tfx™-50 Reagent.
Summary: The reagent was used to transfect WERI-Rb1 human retinoblastoma tissue culture cells.
10. Rodriguez-Viciana, P. *et al.* (1997) Role of phosphoinositide 3-OH kinase in cell transformation and control of the actin cytoskeleton by Ras. *Cell* **89**, 457.
System Used: Tfx™-50 Reagent
Summary: NIH/3T3 cells were stably transfected with either puromycin or G418 selection. Cells were assayed 2 weeks after transfection and selected for foci formation in soft agar.
11. Schenborn, E. and Goiffon, V. (1997) Transfection of insect cells with Tfx™-20 Reagent. *Promega Notes* **63**, 13.
12. Schenborn, E., Oler, J. and Goiffon, V. (1996) A Trio of Tfx™ Transfection Reagents for Eukaryotic Cells. *Promega Notes* **59**, 24.
13. Urban, R.J. and Bodenbun, Y. (1996) Transcriptional activation of the porcine P450 11A insulin-like growth factor response element in MCF-7 breast cancer cells. *J. Biol. Chem.* **271**, 31695.
System Used: Tfx™-50 Reagent.
Summary: The reagent was used to transiently transfect MCF-7 cells.
14. Urban, R.J., Nagamani, M. and Bodenbun, Y. (1996) Tumor necrosis factor α inhibits transcriptional activity of the porcine P45011A insulin-like growth factor response element. *J. Biol. Chem.* **271**, 31699.
System Used: Tfx™-50 Reagent
Summary: The reagent was used to transiently transfect two variants of the NIH/3T3 cells, NWTb3 and KR1.

15. Velcich, A. *et al.* (1997) Organization and regulatory aspects of the human intestinal mucin gene (MUC2) locus. *J. Biol. Chem.* **272**, 7968.

Systems Used: Tfx™-50 Reagent; pGL2 Basic Vector; pGL2 Enhancer Vector; pGL2 Promoter Vector.

Summary: Studies were performed in the intestinal cell lines, HT29 and LS174T, and the HeLa cell line. The Tfx™-50 reagent was used to transfect the intestinal cell lines.

TransFast™ Reagent

1. Schenborn, E., Goiffon, V. and Oler, J. (1998) An efficient new transfection reagent for eukaryotic cells: TransFast™ Reagent. *Promega Notes* **65**, 2.

Transfectam® Reagent for the Transfection of Eukaryotic Cells

1. Carey, D.J., Bendt, K.M. and Stahl, R.C. (1996) The cytoplasmic domain of syndecan-1 is required for cytoskeleton association but not detergent insolubility. Identification of essential cytoplasmic domain residues. *J. Biol. Chem.* **271**, 15253.

Summary: The reagent was used to produce stably transfected Schwann cells.

2. Fisher, E.A. *et al.* (1997) The degradation of apolipoprotein B100 is mediated by the ubiquitin-proteasome pathway and involves heat shock protein 70. *J. Biol. Chem.* **272**, 20427.

Summary: Transfectam® Reagent was used to transfect HepG2 cells.

3. Foley, B.T., Moehring, J.M and Moehring, T.J. (1995) Mutations in the elongation factor 2 gene which confer resistance to diphtheria toxin and *Pseudomonas* exotoxin A. *J. Biol. Chem.* **270**, 23218.

Summary: The reagent was used to produce stably transfected CHO cells.

4. Harada, N. *et al.* (1997) Human IgGF_c binding protein (F_cBP) in colonic epithelial cells exhibits mucin-like structure. *J. Biol. Chem.* **272**, 15232.

Summary: COS-7 cells were transfected with 10µg of DNA and 5µl of Transfectam® Reagent per 500µl of RPMI 1640 media. After 6 hours the medium was changed the cells were cultured for an additional 48 hours prior to assay. CHO cells were plated at 2 x 10⁵ cells per plate 24 hrs prior to transfection as detailed for COS-7 cells. Stably transfected CHO cells were chosen 14 days later after growth in G418.

5. Hinz, M., Moore, M.J. and Bindereif, A. (1996) Domain analysis of human U5 RNA: Cap trimethylation, protein binding and spliceosome assembly. *J. Biol. Chem.* **271**, 19001.

Summary: Transfectam® Reagent was used to transiently transfect 293 cells.

6. Ichijo, H. *et al.* (1997) Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* **275**, 90.

Systems Used: Transfectam® Reagent; Tfx™-50 Reagent.

Summary: Transfectam® Reagent was used to produce stable transfectants of Mv1LU mink lung epithelial cells using hygromycin selection.

7. Ito, M., Jameson, J.L. and Ito, M. (1997) Molecular basis of autosomal dominant neurohypophyseal diabetes insipidus: Cellular toxicity caused by the accumulation of mutant vasopressin presursors within the endoplasmic reticulum. *J. Clin. Invest.* **99**, 1897.

Systems Used: Transfectam® Reagent; CAT Assay System.

Summary: Stable transfectants of Neuro2A cells were generated in G418-containing media. The stable transfectants were assayed for the production of arginine vasopressin and CAT enzyme.

8. Jeannin, P. *et al.* (1997) CD86 (B7-2) on human B cells: A functional role in proliferation and selective differentiation into IgE- and IgG4-producing cells. *J. Biol. Chem.* **272**, 15613.

Summary: Studies were performed in COS cells and the cells were assayed 4 days post-transfection.

9. Kennedy, E.D. *et al.* (1996) Glucose-stimulated insulin secretion correlates with changes in mitochondrial and cytosolic Ca²⁺ in aequorin-expressing INS-1 cells. *J. Clin. Invest.* **98**, 2524.

Summary: INS-1 cells were stably transfected with a neomycin-resistant plasmid and an aequorin expression vector. The cells were assayed by immunocytochemistry, various assays of insulin secretion and Ca²⁺ channel activity.

10. Kizer, N., Guo, X.-L. and Hruska, K. (1997) Reconstitution of stretch-activated cation channels by expression of the α -subunit of the epithelial sodium channel cloned from osteoblasts. *Proc. Natl. Acad. Sci. USA* **94**, 1013.

Summary: LM(TK⁻) mouse cells were stably transfected and selected in media containing hygromycin. The resulting cells were analyzed by Northern blot, Western blot and patch-clamp protocols.

11. Klafki, H.-W. *et al.* (1996) The carboxyl termini of β -amyloid peptides 1-40 and 1-42 are generated by distinct γ -secretase activities. *J. Biol. Chem.* **271**, 28655.

Summary: The reagent was used to transiently transfect COS-1 cells.

12. Lesch, K.-P. *et al.* (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* **274**, 1527.
Systems Used: Transfectam® Reagent; pGL3 Basic Vector; pGL3 Control Vector; pSV-β-Galactosidase Control Vector.
Summary: Studies were performed in human lymphoblasts. For transient expression, lymphoblasts (2×10^5 cells) were exposed for 24 hours to 5 μg of construct DNA complexed with 5 μl of Transfectam® Reagent in 5 ml of RPMI 1640. Cells were grown an additional 24 hours, then assayed for luciferase and β-galactosidase activity.
13. Nemoto, Y. *et al.* (1996) Regulatory function of delta/YY-1 on the locus control region-like sequence of mouse glycophorin gene in erythroleukemia cells. *J. Biol. Chem.* **271**, 13542.
Summary: Transfectam® Reagent was used to transfect MEL Murine Erythroleukemia cells.
14. Nibbs, R.J.B. *et al.* (1997) Cloning and characterization of a novel murine β chemokine receptor, D6: Comparison to three other related macrophage inflammatory protein-1α receptors, CCR-1, CCR-3 and CCR-5. *J. Biol. Chem.* **272**, 12495.
Summary: The Transfectam® Reagent was used to generate G418-resistant, stably-transfected HEK293 cells.
15. Olofsson, A. *et al.* (1995) Efficient association of an amino-terminally extended form of human latent transforming growth factor-β binding protein with the extracellular matrix. *J. Biol. Chem.* **270**, 31294.
Summary: Transfectam® Reagent was used to transiently transfect COS-1 cells.
16. Rosoff, M.L., Wei, J. and Nathanson, N.M. (1996) Isolation and characterization of the chicken m2 acetylcholine receptor promoter region: Induction of gene transcription by leukemia inhibitory factor and ciliary neurotrophic factor. *Proc. Natl. Acad. Sci. USA* **93**, 14889.
Systems Used: Transfectam® Reagent; pGL3 Basic Vector; pSV-β-Galactosidase Control Vector.
Summary: Transfectam® Reagent was used at 3.5 μl/μg of DNA to transfect primary chicken heart cultures. The DNA/ Transfectam® solution was left in contact with the cells for 6-7 hours. Thirty hours post-transfection, the cells were lysed and assayed for luciferase and β-galactosidase activity.
17. Smit, M.J. *et al.* (1996) Two distinct pathways for histamine H₂ receptor down-regulation: H₂ Leu¹²⁴-αAla receptor mutant provides evidence for a cAMP-independent action of H₂ agonists. *J. Biol. Chem.* **271**, 7574.
Summary: The reagent was used to stably transfect CHO cells.
18. Venkatakrishnan, G. and Exton, J.H. (1996) Identification of determinants in the α-subunit of Gq required for phospholipase C activation. *J. Biol. Chem.* **271**, 5066.
Summary: Transfectam® Reagent was used to transiently transfect 293 cells.
19. Zhang, L., David, G. and Esko, J.D. (1995) Repetitive Ser-Gly sequences enhance heparan sulfate assembly in proteoglycans. *J. Biol. Chem.* **270**, 27127.
Summary: Transfectam® Reagent was used to produce stably transfected CHO cells.

Profection® Mammalian Transfection System-CaPO₄

1. Behrooz, A. and Ismail-Beigi, F. (1997) Dual control of *glut1* glucose transporter gene expression by hypoxia and by inhibition of oxidative phosphorylation. *J. Biol. Chem.* **272**, 5555.
Systems Used: ProFection® Mammalian Transfection System -CaPO₄; Dual-Luciferase™ Reporter Assay System; pGL2 Basic Vector; pRL-TK Vector; pCAT® Control Vector.
Summary: The *Renilla* luciferase activity was used to normalize the firefly luciferase activity of transfected Clone 9 cells.
2. Chu, B. *et al.* (1996) Sequential phosphorylation by mitogen-activated protein kinase and glycogen synthase kinase 3 represses transcriptional activation by heat shock factor-1. *J. Biol. Chem.* **271**, 30847.
Summary: NIH/3T3 cells were seeded at 2.5×10^5 cells/100mm dish 24 hours prior to transfection. Cells were assayed 48 hours post-transfection.
3. Hoock, T.C., Peters, L.L. and Lux, S.E. (1997) Isoforms of ankyrin-3 that lack the NH₂-terminal repeats associate with mouse macrophage lysosomes. *J. Cell Biol.* **136**, 1059.
Summary: The ProFection® Mammalian Transfection System was used to transiently transfect COS cells. The DNA precipitates were left in contact with the cells for 16 hours and 48 hours later the cells were analyzed by immunofluorescence.
4. Obiri, N.I. *et al.* (1997) Modulation of interleukin (IL)-13 binding and signaling by the γ_c chain of the IL-2 receptor. *J. Biol. Chem.* **272**, 20251.
System Used: ProFection® Mammalian Transfection System -CaPO₄
Summary: The system was used to produce stable transfectants in the ML-RCC renal carcinoma cell line.

5. Walsh, A.A. *et al.* (1996) Identification of a novel *cis*-acting negative regulatory element affecting expression of the CYP1A1 gene in rat epidermal cells. *J. Biol. Chem.* **271**, 22746.

Systems Used: ProFection® Mammalian Transfection System -CaPO₄; pGL2 Basic Vector; Luciferase Assay System.

Summary: Studies were performed in rat epidermal keratinocytes. Cells were grown to 50% confluency in 60mm dishes. Cells were transfected with 15µg of plasmid DNA for 24 hours then the media was replaced and the cells assayed 24 hours later.

Profection® Mammalian Transfection System-DEAE-Dextran

1. Pierce, R.A. *et al.* (1996) Monocytic cell type-specific transcriptional induction of collagenase. *J. Clin. Invest.* **97**, 1890.

Summary: U-937 cells (1 x 10⁷ cells) were transfected in suspension with 10µg of DNA and 75µg of DEAE-Dextran for 20 min at room temperature. The cells were divided and allowed to recover overnight. The cells were treated with a reagent and CAT activity assayed 24 hours later.

2. Rohlf, C. *et al.* (1997) Modulation of transcription factor Sp1 by cAMP-dependent protein kinase. *J. Biol. Chem.* **272**, 21137.

Systems Used: ProFection® Mammalian Transfection System-DEAE-Dextran; pCAT® Basic Vector; pCAT® Promoter Vectors, Recombinant Human Sp1.

Summary: Reporter studies were performed in HL-60 and HL-60/AR (doxorubicin-resistant isolate) leukemia cells. DNA was transfected by electroporation in the presence of DEAE-Dextran and many details of the transfection are reported. Recombinant Sp1 was *in vitro* phosphorylated with the cAMP dependent protein kinase catalytic subunit and used in gel shift assays.

3. Zhang, M., Magit, D and Sager, R. (1997) Expression of maspin in prostate cells is regulated by a positive Ets element and a negative hormonal responsive element site recognized by androgen receptor. *Proc. Natl. Acad. Sci. USA* **94**, 5673.

Summary: LNCaP human prostate tumor cells, CF3 normal human epithelial cells and 70N mammary epithelial cells were transfected at 75% confluency in a 100mm dish. Cells were assayed 48 hours post-transfection for β-gal and CAT activity.

References Using Promega's Reporter Assay Systems and Expression Vectors

References using Promega's reporter assay systems and expression vectors are available on the Internet at www.promega.com.