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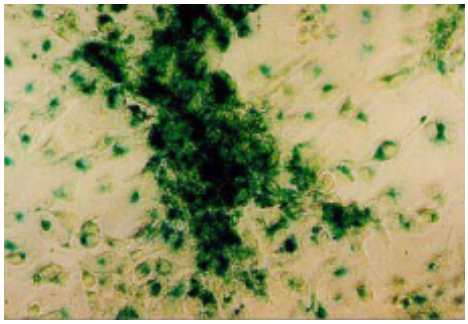
Targeting Systems Introduces :

Targefect-Cos



Cos cells transfected with Targefect-Cos :

Transfection efficiency is approximately 80%.

Targefect-Cos Transfection Reagent is specifically developed to achieve the highest transfection efficiency in Cos cells.

Transfection protocol for transfection in presence of serum

Set up cells to be transfected so that they are about 70-80% confluent at the time of the experiment. Plate out or maintain cells to be transfected in media with 5% serum. This protocol has been optimized for cells maintained in media with 5% serum. Higher serum concentrations may need adjustment of transfection conditions (i.e. DNA: targefect ratios may need to be changed).

Prepare transfection complexes as follows :

Set up cells to be transfected so that they are about 70-80% confluent at the time of the experiment.

Prepare transfection complexes as follows:

Note : It is important to use high glucose DMEM (DMEM containing 4500 mg/liter glucose for complex formation. Use clear plastic tubes for complex formation. Do not vortex the Targefect-Cos reagent. Thaw the reagent if it arrives frozen and gently mix it by inverting the tube several times and then store at 4 oC. Do not freeze this reagent. Prepare transfection complexes as follows if you are performing duplicate transfections in 35 mm dishes/6-well plates.

Tube #	DMEM	Plasmid DNA	Targefect-Cos
1	0.5 ml	6 µg	12 µl

Add DMEM 1 first, then add DNA, mix well by flicking the tube about 12 times to create a vortexing action. Add targefect next, mix well again by flicking the tube. Incubate the tubes at 37oC for 20 minutes to form the transfection complexes. Add the appropriate amount of transfection complex per well/dish. Swirl the dish and incubate at 37 o C overnight. Assay at 24-48 hrs post transfection.

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Recommended volumes of transfection complex for performing transfection in different size dishes :

Culture Vessel	Volume of plating medium	DNA (µg) in transfection complex volume (µl)	Targefect in transfection complex volume (µl)
96well	100 µl	0.2 µg in 25 µl	0.5 µl in 25 µl
24well	500 µl	0.8 µg in 50 µl	1.5 µl in 50 µl
12well	1 ml	1.6 µg in 150 µl	3.5 µl in 150 µl
35mm	2 ml	3.0 µg in 250 µ	6 µl in 250 µl
6well	2 ml	3.0µg in 250 µl	6 µl in250 µl
60mm	5 ml	6.0 µg in 0.5 ml	12 µl in 0.5ml
10cm	15 ml	18 µg in 1.5 ml	36 µl in 1.5 ml

Note: The above conditions are standardized using media with 5% FBS.

List of publications citing use of Targefect reagents for transfection of Cos-cells

1) Xiaoyong Yang, Fengxue Zhang, and Jeffrey E. Kudlow
Recruitment of O-GlcNAc Transferase to Promoters by Corepressor mSin3A: Coupling Protein O-GlcNAcylation to Transcriptional Repressio. Cell 2002 110: 69-80.

2) Marc Tini, Arndt Benecke, Soo-Joong Um, Joseph Torchia, Ronald M. Evans, and Pierre Chambon (2002) Association of CBP/p300 Acetylase and Thymine DNA Glycosylase Links DNA Repair and Transcription. Molecular Cell 9: 265-277.

3) Hongwei Li, Sungmin Park, Britta Kilburn, Mary Anne Jelinek, Agnes Henschen-Edman, Dana W. Aswad, Michael R. Stallcup, and Ite A. Laird-Offringa Lipopolysaccharide-induced Methylation of HuR, an mRNA-stabilizing Protein, by CARM1. J. Biol. Chem., Nov 2002; 277: 44623 - 44630.

4) Nicholas Grammatikakis, Jun-Hsiang Lin, Aliki Grammatikakis, Philip N. Tsichlis, and Brent H. Cochran p50cdc37 Acting in Concert with Hsp90 Is Required for Raf-1 Function Mol. Cell. Biol., Mar 1999; 19: 1661 - 1672.

5) Takako Kitani, Sachiko Okuno, Masayuki Takeuchi, and Hitoshi Fujisawa Subcellular distributions of rat CaM kinase phosphatase N and other members of the CaM kinase regulatory system J. Neurochem., Jul 2003; 86: 77 - 85.

6) Stephen S. Koh, Hongwei Li, Young-Ho Lee, Randall B. Widelitz, Cheng-Ming Chuong, and Michael R. Stallcup Synergistic Coactivator Function by Coactivator-associated Arginine Methyltransferase (CARM) 1 and -Catenin with Two Different Classes of DNA-binding Transcriptional Activators J. Biol. Chem. 2002 277: 26031-26035.

7) Young-Ho Lee, Stephen S. Koh, Xing Zhang, Xiaodong Cheng, and Michael R. Stallcup Synergy among Nuclear Receptor Coactivators: Selective Requirement for Protein Methyltransferase and Acetyltransferase Activities Mol. Cell. Biol., Jun 2002; 22: 3621-3632.

8) Radu V. Stan, Eugene Tkachenko, and Ingrid R. Niesman (2004) PV1 Is a Key Structural Component for the Formation of the Stomatal and Fenestral Diaphragms Mol. Biol. Cell, Aug 2004; 15: 3615-3630.

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9) Radu V. Stan (2004) Multiple PV1 dimers reside in the same stomatal or fenestral diaphragm. Am J Physiol Heart Circ Physiol, Apr 2004; 286: 1347 - 1353.

10) Nary Veal, Chih-Lin Hsieh, Shigang Xiong, Jose M. Mato, Shelly Lu, and Hidekazu Tsukamoto (2004) Inhibition of lipopolysaccharide-stimulated TNF- promoter activity by S-adenosylmethionine and 5'-methylthioadenosine. Am J Physiol Gastrointest Liver Physiol, Aug 2004; 287: 352 - 362.

11) Young-Ho Lee, Scott A. Coonrod, W. Lee Kraus, Mary Anne Jelinek, and Michael R. Stallcup Regulation of coactivator complex assembly and function by protein arginine methylation and demethylimination. PNAS, Mar 2005; 102: 3611-3616.

12) Young-Ho Lee, Scott A. Coonrod, W. Lee Kraus, Mary Anne Jelinek, and Michael R. Stallcup (2005) Regulation of coactivator complex assembly and function by protein arginine methylation and demethylimination PNAS, Mar 2005; 102: 3611 - 3616.

13) Harald Wodrich, Tinglu Guan, Gino Cingolani, Dan Von Seggern, Glen Nemerow, and Larry Gerace (2003) Switch from capsid protein import to adenovirus assembly by cleavage of nuclear transport signals EMBO J., Dec 2003; 22: 6245 - 6255.

Related cell types : For CV1 cells use the targefect reagent

1) Michael Downes, Peter Ordentlich, Hung-Ying Kao, Jacqueline G. A. Alvarez, and Ronald M. Evans. Identification of a nuclear domain with deacetylase activity PNAS, Sep 2000; 97:10330-10335.

2) Hung-Ying Kao, Michael Downes, Peter Ordentlich, and Ronald M. Evans Isolation of a novel histone deacetylase reveals that class I and class II deacetylases promote SMRT-mediated repression. Genes & Dev., Jan 2000; 14: 55 - 66.

3) Young-Ho Lee, Stephen S. Koh, Xing Zhang, Xiaodong Cheng, and Michael R. Stallcup (2002) Synergy among Nuclear Receptor Coactivators: Selective Requirement for Protein Methyltransferase and Acetyltransferase Activities Mol. Cell. Biol., 22: 3621 3632

Product No.	Quantity	T.S. list Price
#COS-01	1.5 ml	$170
#COS-10	10 x 1.5 ml	$1600

Contents : Single solution

Targefect - Cos