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Targeting Systems Introduces :

Targefect-Hepatocyte

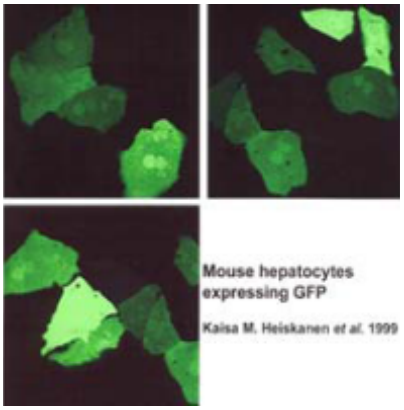

Figure 1 :
Mouse hepatocytes transfected with Targefect-Hepatocyte plus enhancer : Data courtesy of Dr. K. Haiskanen and Dr. A. Nieminen Case Western Reserve University, Cleveland OH

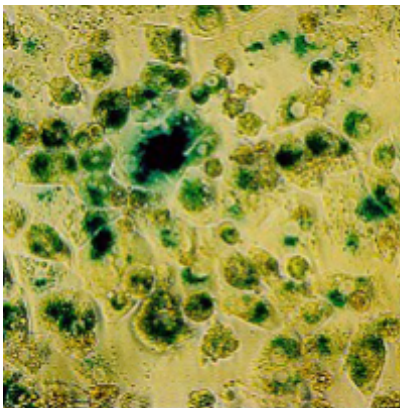

Figure 2 :
Human hepatocytes transfected with Targefect-Hepatocyte : Targeting Systems, CA

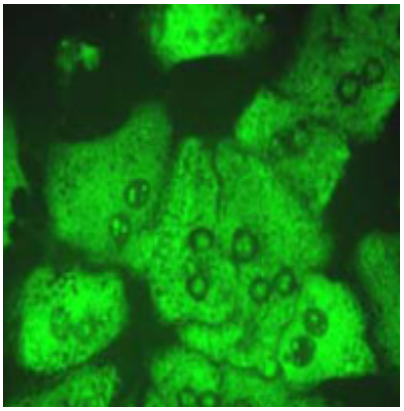

Figure 3 :
Mouse hepatocytes transfected with Targefect-Hepatocyte :
Data courtesy of Dr. Suzanne Lyman, Dr. Behren's lab,University of North Carolina at Chapell Hill, NC

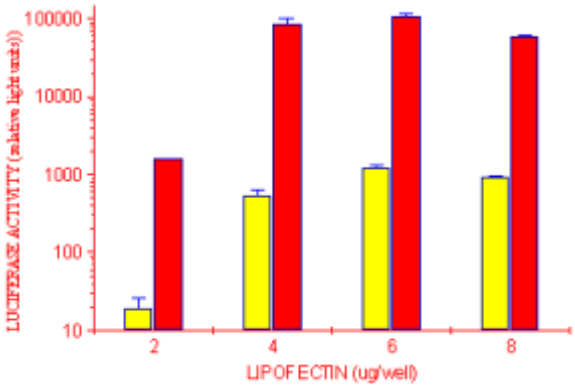

Figure 4 :
Enhancement of gene transfer into primary rat Hepatocytes using Virofect in combination with another commercial cationic lipid-lipofectin (GIBCO BRL)
Note: Virofect can be used with Targefect as well as other cationic reagents to enhance gene transfer

The Targefect-Hepatocyte reagent is a combination of the Targefect-Hepatocyte reagent with the Virofect enhancer for optimal transfection of primary Hepatocytes.

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PROTOCOL for transfecting primary Rat, Mouse, Human or Chimp Hepatocytes.

General considerations :

We recommend plating out hepatocytes on Primaria (Falcon) or Cellbind (Corning) tissue culture dishes. Plating out hepatocytes on matrigel dishes and then transfecting them results in lower transfection efficiency. If you do want to use matrigel you should transfect hepatocytes first and then add the matrigel.
Written below are two recommended protocols (the general transfection protocol and a second transfection protocol which enables transfection in presence of serum). It is also possible to transfect hepatocytes in suspension prior to plating. At the end of this file is a list of publications citing use of the targefect reagent for hepatocytes transfections.

General transfection protocol :

Thaw and vortex the Targefect-Hepatocyte reagent at full speed for 30 seconds once just before starting complex formation.
Set up cells to be transfected so that they are about 70 % confluent the day of the experiment.
Prepare transfection complexes as follows using clear 15 ml conical tubes for complex formation. Remember to thaw and vortex the Targefect-Hepatocyte reagent at full speed for 30 seconds before use.
Note: Please use high glucose DMEM (serum free DMEM with 4500 mg / liter glucose) as the complexing medium.

Tube #	DMEM	DNA	Targefect-Hepatocyte
1	1ml	2µg	2.5µl
2	1ml	2µg	5µl
3	1ml	2µg	7.5ul
4	1ml	1µg	2.5µl

Note : We recommend first trying the Targefect-Hepatocyte reagent alone as this gives good transfection efficiencies. Some labs have reported enhancement of gene transfer using Virofect in addition to the Targefect-Hepatocyte. When using Virofect add 10µl of or 20µl of Virofect to 2µg DNA complexed with .5µl of F-1 in 1ml DMEM. The efficiencies are high even without virofect.

Add DMEM first, and then add DNA mix well by flicking the tube with your hand about 12 times to create a vortexing action, add Targefect-Hepatocyte, and mix well again. Incubate at 37°C for 20 mins to permit complex formation.
Wash cells to be transfected with DMEM twice, aspirate second wash completely and add 1 ml of the transfection complex per 35 mm dish, 250ul of transfection complex per well of a 12-well dish or 150ul of transfection complex per well of a 24-well dish (make sure that the complexes cover cells well.). Incubate 37° C for 2-3 hrs. Add 2 ml of complete media with serum for a 6-well dish (1ml for a 12-well or 24-well dish). Incubate at 37°C (CO2 incubator) and assay at 24-48 hrs post transfection.

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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 :

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. Vortex the Targefect-Hepatocyte reagent thaw the reagent and vortex at full speed for 30 seconds once just before preparing the transfection complexes.
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 37oC overnight. Assay at 24-48 hrs post transfection.

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 1 : The above conditions are standardized using media with 5% fetal bovine serum. The transfection protocols in the presence of serum have been tested in HEK-293 cells, Cos, CV-1 cells, NIH 3T3 cells and HUVECs.

Note 2 : We recommend adding 50ul of virofect enhancer per ml of the transfection Complex. The virofect enhancer should be added immediately after addition of Targefect. The above conditions are standardized using media with 5% fetal bovine serum.

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Publications citing use of targefect reagents for transfecting primary hepatocytes

1) Seung-Hoi Koo, Hiroaki Satoh, Stephan Herzig, Chih-Hao Lee, Susan Hedrick, Rohit Kulkarni, Ronald M Evans, Jerrold Olefsky, Marc Montminy. PGC-1 promotes insulin resistance in liver through PPAR--dependent induction of TRB-3 Nature Medicine10, 530 -534 (01 May 2004) Letters.

2) Koo SH and Towle HC (2000) Glucose regulation of mouse S-14 gene expression in hepatocytes- involvement of a novel transcription factor complex. J. Biol. Chem 275(7) : 5200-5207

3) Johnson SAS, Mandavia N, Wang HD, and Johnson DL (2000) Transcriptional regulation of the TATA-binding protein by Ras cellular signaling. Mol. Cell. Biol. 20 (14): 5000-9.

4) Koo SH, Dutcher AK and Towle HCl (2001). Glucose and insulin function through two distinct transcription factors to stimulate expression of lipogenic enzymes in liver. J. Biol. Chem. (275(10): 1074

5) Brennon L. O'Callaghan, Seung-Hoi Koo, Yue Wu, Hedley C. Freake, and Howard C Towle Glucose Regulation of the Acetyl-CoA Carboxylase Promoter PI in Rat Hepatocytes J. Biol. Chem. 2001 276: 16033-16039

6) Etsuro Hatano and David A. Brenner (2001)Akt protects mouse hepatocytes from TNF- and Fas-mediated apoptosis through NK- B activation Am J Physiol Gastrointest Liver Physiol, Dec 2001; 281: 1357 - 1368.

7) Robert F. Schwabe, Cynthia A. Bradham, Tetsuya Uehara, Etsuro Hatano, Brydon L. Bennett , Robert Schoonhoven , David A. Brenner (2003) c-Jun-N-terminal kinase drives cyclin D1 expression and proliferation during liver regeneration. Hepatology. Volume 37 • Number 4 • p824 to p832

8) Angela K. Stoeckman, Lin Ma, and Howard C. Towle (2004) Mlx Is the Functional Heteromeric Partner of the Carbohydrate Response Element-binding Protein in Glucose Regulation of Lipogenic Enzyme Genes. J. Biol. Chem., 279: 15662 - 15669.

9) Lin Ma, Nikolas G. Tsatsos, and Howard C. Towle (2005) Direct Role of ChREBP·Mlx in Regulating Hepatic Glucose-responsive Genes J. Biol. Chem., Mar 2005; 280: 12019 - 12027.

10) Elizabeth N. Kaytor, Juan Li Zhu, Ching-I Pao, and Lawrence S. Phillips Insulin-responsive Nuclear Proteins Facilitate Sp1 Interactions with the Insulin-like Growth Factor-I Gene. J. Biol. Chem., Sep 2001; 276: 36896 - 36901.

11) B. Amirimani, B. Ning, A.C. Deitz, B.L. Weber, F.F. Kadlubar, T.R. Rebbeck Increased transcriptional activity of the CYP3A4*1B promoter variant (2003) Environmental and Molecular Mutagenesis. Volume 42, Issue 4, .Pages 299-305

12) Betty C. Villafuerte and Elizabeth N. Kaytor (2005) An insulin-response element binding protein that ameliorates hyperglycemia in diabetes. J. Biol. Chem., Mar 2005; 10.1074/jbc.M410817200.

13) Magnana MM, Koo S-H, Towle HC and Osborne TF (2000) Different sterol regulatory element-binding protein-1 isoforms utilize distinct co-regulatory factors to activate the promoter for fatty acid synthase. J. Biol. Chem. 275(7): 4726-4733

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Related cell types (transection of hepatoma cell lines): References citing use of targefect for hepatoma cell lines:

1) Keyvan Mahboubi and Jordan S. Pober Activation of Signal Transducer and Activator of Transcription 1 (STAT1) Is not sufficient for the Induction of STAT1-dependent Genes in Endothelial Cells. COMPARISON OF INTERFERON- AND ONCOSTATIN M J. Biol. Chem.2002 277: 8012-8021.

2) Elizabeth N. Kaytor, Juan Li Zhu, Ching-I Pao, and Lawrence S. Phillips (2001). Insulinresponsive nuclear proteins facilitate Sp1 interactions with the IGF-I gene J. Biol.Chem. published July 16, 2001 as 10.1074/jbc.M104035200

3) Hailing Liao, Thomas Langmann, Gerd Schmitz, and Yi Zhu (2002) Native LDL Upregulation of ATP-Binding Cassette Transporter-1 in Human Vascular Endothelial Cells Arterioscler. Thromb. Vasc. Biol., Jan 2002; 22: 127 - 132.

For improved transfection of primary hepatocytes and hepatoma lines we recommend using the virofect enhancer in combinaiton with targefect.

This kit (Catalog # HEP-01) contains sufficient targefect reagent for performing 200 transfections in 12-well dishes or 100 transfections in 6-well dishes

Targefect-Hepatocyte Catalog #	Quantity	Price
HEP-01	1	$250
HEP-10	10	$2,000

Contents : Two solutions

Targefect-Hepatocyte and Virofect (600ul each)