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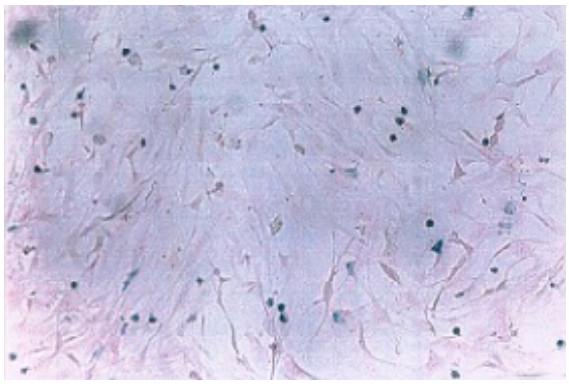
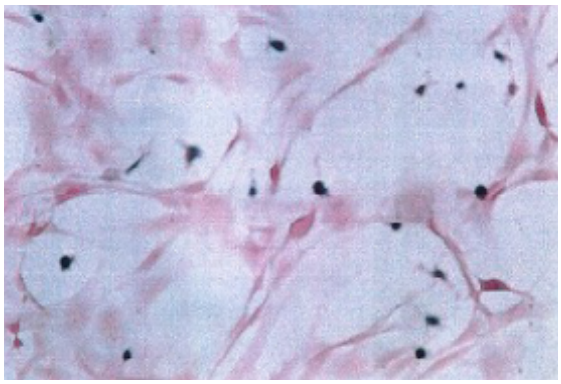
Targeting Systems Introduces :

Targefect-HSC

For transfection of primary hepatic stellate cells and stellate cell lines.





Transfection of rat liver stellate cells with Targefect-HSC

Rat liver stellate cells were transfected with a reporter construct pSV40-ßgal, expressing the the Lac-Z reporter gene under control of the SV40 promoter using Targefect-HSC under the optimal conditions determined from a previous experiment. The cells were fixed and stained for ß-galactosidase expression 48 hours post transfection. (Data have been kindly provided by Dr. Carl Holgum, UCSD, San Diego)

Transfection Protocol for stellate cells

Set up cells to be transfected so that they are about 70% confluent at the time of the experiment. Please use OptiMEM 1 for forming transfection complexes.
Store the targefect-HSC reagent at 4º C. Do not vortex this reagent.

Prepare transfection complexes as follows : Use clear plastic tubes for complex formation.

Tube #	OptiMEM 1	DNA	Targefect-HSC
1	1 ml	2 µg	2 µl
2	1 ml	2 µg	4 µl
3	1 ml	1 µg	7 µl

Add Optimem 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. If using an enhancer add it after adding the targefect reagent. Incubate the tubes at 37° C for 25 minutes to form the transfection complexes. The amount of enhancer varies depending on the cell type we recommend trying 5 µl, 10 µl and 25 µl of the enhancer per 2 µg DNA.

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Wash cells to be transfected twice with Opitmem 1 . Aspirate the second wash completely. Add 1 ml of the transfection mix for 1 well for a 6-well dish (or for a 35 mm dish). Prepare 2 ml of transfection complex per 60 mm dish and 4 ml of transfection complex per 100mm dish. Incubate the transfection complexes with the cells at 37° C for 3 hrs. Add Complete media with serum (1 ml for a 35 mmm dish, 2 ml for a 60 mm dish and 6 ml for a 10 cm dish. Incubate overnight. Replace the media with fresh complete media the next morning and assay at 36-48 hrs post-transfeciton.

Protocl for transfecting stellate cells in the presence of serum :

Tube #	High glucose DMEM	DNA	Targefect-HSC
3	0.5 ml	6 µg	6 µl
4	0.5 ml	6 µg	12 µl

• Add DMEM first, mix well, then add targefect, mix well, Add enhancer last and mix well again . Incubate at 37 oC to form transfection complexes. Add 70 ul of transfection complex to 0.25 ml of complete media per well of a 24 well dish
• Add 125 ul of transfection complex to 0.5 ml of complete media per well of a 12 well dish
• Add 250 ul of transfection complex to 1ml of complete media per well of a 6 well dish.

Swirl the dish to mix transfection complexes with complete media. Replace media with complete media the next morning. Assay at 30-48 hrs post transfection.

Product No.	Quantity	T.S. list Price
HSC-01	1 ml	$170
HSC-10	10ml	$1600

Contents : Single solution

Targefect - HSC (1 ml)

Publications citing use of Targefect for transfecting liver stellate cells

1) Chin K. Sung, Hongyun She, Shigang Xiong, and Hidekazu Tsukamoto Tumor necrosis factor- inhibits peroxisome proliferator-activated receptor activity at a posttranslational level in hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol, May 2004; 286: 722 - 729.

2) Saswati Hazra, Shigang Xiong, Jiaohong Wang, Richard A. Rippe, V. Krishna, K. Chatterjee, and Hidekazu Tsukamoto (2004) Peroxisome Proliferator-activated Receptor Induces a Phenotypic Switch from Activated to Quiescent Hepatic Stellate Cells. J. Biol. Chem., 279: 11392 - 11401.