

Targeting Systems Introduces :
Targefect-PCL

For transfection of pancreatic cell lines and primary islet cells

Transfection of INS-1 and MIN-6 cells with the Targefect-PCL reagent.

Transfection was performed as follows :

Perform transfections in 24 well plates.

Complex 12 µg DNA with 48 µl Targefect-PCL and 48 µl of Virofect in 1.2 ml DMEM. Add 0.05 ml complex to 0.5 ml of complete media in one well of a 24 well dish.

Transfection efficiency approx 80% in MIN-6 cells.

Transfection efficiency 50% in INS-1 cells

Add complexes to cells immediately after plating in 24-well dishes (plated to get approx 30% confluence).

A transfection efficiency of 60-80% was achieved in various pancreatic cell lines using this protocol. Data courtesy of Dr. Dongmin Liu, Department of Nutrition, Virginia Tec, Blacksburgh, VA

Suggested Transfection protocol

The Targefect-PCL kit is a combination of the Targefect-PCL reagent with the Virofect enhancer for optimal transfection of primary islet cells. Set up cells to be transfected so that they are about 70% confluent at the time of the experiment.

Prepare transfection complexes as follows :

- Use clear plastic tubes for complex formation. Vortex Targefect-PCL at full speed for 30 seconds before use.

Complexing conditions for formation of transfection complexes :

- Use high glucose DMEM (Dulbecco's modified eagle's medium containing 4500 mg/liter glucose).

Tube #	High Glucose DMEM	DNA	Targefect-PCL	Virofect
1	0.5 ml	6 µg	12 µl	-
2	0.5 ml	6 µg	12 µl	25 µl
3	0.5 ml	4 µg	12 µl	25 µl

Add DMEM 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. Add Virofect last and mix again. Incubate the tubes at 37oC for 25 minutes to form the transfection complexes.

For a 12-well dish add 125 µl of transfection complex to 1 ml of complete media per well.

Add 0.25 ml of the transfection mix to 2 ml of complete media for 1 well for a 6-well dish (or for a 35 mm dish). Prepare 0.5 ml of transfection complex per 60 mm dish and 1.25 ml of transfection complex per 100mm dish. Swirl the dish to mix transfection complexes with the cell culture media (with 5-10% serum). Incubate overnight at 37° C in CO2 incubator. Aspirate media containing transfection complexes 3 hrs after transfection and replace with complete growth media

Virofect enhancer : Please store at -70° C on arrival

Targefect- PCL : Store at -20 o C or at -70 oC. Please thaw this reagent and vortex it at full speed for 30 seconds once or twice just before use.

Expected efficiency – 60-80%. This kit (catalog # PCL-01) contains sufficient reagent for performing 200 transfections in 12-well dishes or 100 transfections in 6-well dishes. Cell types tested: INS-1, MIN-6, Capan-1, primary rat islet cells.

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

Contents : Two solutions

Targefect-PCL (1 ml) and Virofect (600 µl)

Cell lines tested: Capan-1, MIN-6, INS-1, primary rat islet cells

References citing use of Targefect reagents for pancreatic cells

1) Hella Wobser, Heiko Düßmann, Donat Kögel, Haiyan Wang, Claus Reimertz, Claes B. Wollheim, Maria M. Byrne, and Jochen H. M. Prehn (2002) Dominant-negative Suppression of HNF-1 Results in Mitochondrial Dysfunction, INS-1 Cell Apoptosis, and Increased Sensitivity to Ceramide-, but Not to High Glucose-induced Cell Death J. Biol. Chem. 277: 6413-6421.