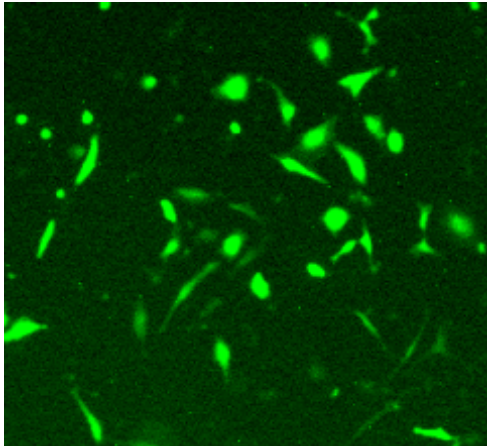


Targeting Systems Introduces :

Targefect-Cos

A modification of the targefect reagent protocol for optimal transfection into HeLa cells in the presence or absence of serum



Transfection Protocol HeLa Cells

HeLa cells transfected with a green fluorescent protein expression vector using the Targefect-Hela reagent :

Data courtesy of Dr. Radu Stan-Vigil, University of California at San Diego, San Diego, CA

Transfection Protocol

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. Do not vortex the Targefect-Hela reagent. Thaw the reagent if it arrives frozen and gently mix it by inverting the tube several times and then store at 4 °C. Do not freeze this reagent.

Tube #	DMEM	Plasmid DNA	Targefect-Hela
1	0.6 ml	6 µg	12 µl
2	0.6 ml	6 µg	6 µl

Add DMEM 1 first, then add DNA, mix well by flicking the tube about 12 times to create a vortexing action. Add Targefect-Hela next, mix well again by flicking the tube. Incubate the tubes at 37° C for 20 minutes to form the transfection complexes. Add 250ul of transfection complex to 2 ml of complete media (media with 10% serum) per well of a 6-well dish. Swirl the dish to mix transfection complexes with complete media and incubate at 37° C overnight. Assay at 24-48 hrs after 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-Hela 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 μl	6 μl in 250 μl
6well	2 ml	3.0 μg in 250 μl	6 μl in 250 μl
60mm	5 ml	6.0 μg in 0.5 ml	12 μl in 0.5 ml
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% fetal bovine serum.

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

**Contents : Single solution
 Targefect - Hela (1 ml)**

Publications using Targefect for transfecting Hela cells

- 1) Vadim Iourgenko, Wenjun Zhang, Craig Mickanin, Ira Daly, Can Jiang, Jonathan M. Hexham, Anthony P. Orth, Loren Miraglia, Jodi Meltzer, Dan Garza, Gung-Wei Chirn, Elizabeth McWhinnie, Dalia Cohen, Joanne Skelton, Robert Terry, Yang Yu, Dale Bodian, Frank P. Buxton, Jian Zhu, Chuazheng Song, and Mark A. Labow (2003) Identification of a family of cAMP response element-binding protein coactivators by genome-scale functional analysis in mammalian cells. PNAS, Oct 2003; 100: 12147 - 12152
- 2) Radu V. Stan, Eugene Tkachenko, and Ingrid R. Niesman used F-2 PV1 Is a Key Structural Component for the Formation of the Stomatal and Fenestral Diaphragms Mol. Biol. Cell, Aug 2004; 15: 3615 - 3630.
- 3) Radu V. Stan Multiple PV1 dimers reside in the same stomatal or fenestral diaphragm/ Am J Physiol Heart Circ Physiol, Apr 2004; 286: 1347 - 1353.