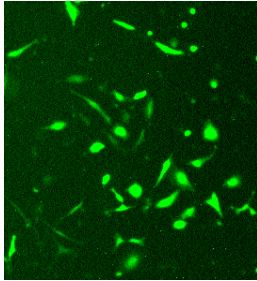


Targeting Systems Introduces:

Targefect-Hela -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, Sand 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	0.6ml	6ug	12ul
2	0.6ml	6ug	6ul

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 (ug) in transfection complex volume(ul)	Targefect-Hela in transfection complex volume (ul)
96well	100ul	0.2ug in 25ul	0.5ul in 25ul
24well	500ul	0.8ug in 50ul	1.5ul in 50ul
12well	1ml	1.6ug in 150ul	3.5ul in 150ul
35mm	2ml	3.0ug in 250ul	6ul in 250ul
6well	2ml	3.0ug in 250ul	6ul in 250ul
60mm	5ml	6.0ug in 0.5ml	12ul in 0.5ml
10cm	15ml	18ug in 1.5ml	36ul in 1.5ml

Note: The above conditions are standardized using media with 5% fetal bovine serum.

Product No.	Quantity	Price
Hela-01	1ml	\$170
Hela-10	10 x 1ml	\$1,600

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, Chuangzheng 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.