targetingsystems

1453 N. Cuyamaca St, El Cajon, CA 92020
Phone: (866)-620 4018, (619)-562-1518
Fax: (619)-562-1326 • info@targetingsystems.net
www.targetingsystems.com

Targeting Systems Introduces :

Targefect-MEF

For transfection of Primary Mouse Embryo Fibroblasts and fibroblast cell lines.

Transfection of primary Mouse Embryo Fibroblasts using the Targefect MEF transfection kit. Transfection efficiency approx 50%. Data courtesy of Dr. Erika Brown, Medical University of South Carolina, Charleston, SC

Cell types tested

Primary Mouse Embryo fibroblasts, rat fibroblasts, primary human skin fibroblasts, HS-27 cells (human fibroblast cell line) and rat fibroblast cell lines. Transfection efficiency in human fibroblasts is very low (approx 10-15%)

Suggested Transfection protocol

The Targefect-MEF kit is a combination of the Targefect-MEF reagent and the Virofect enhancer for optimal transfection of primary Mouse Embryo fibroblasts and fibroblast cell lines.

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.
• Complexing conditions for formation of transfection complexes:
• Use high glucose DMEM (Dulbecco's modified eagle's medium containing 4500 mg/liter glucose). Please thaw the Targefect-MEF reagents and vortex it at full speed for 30 seconds once or twice just before use.

Tube #	High glucose DMEM	DNA	Targefect-MEF	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 oC in CO2 incubator. Aspirate media containing transfection complexes 3 hrs after transfection and replace with complete growth media.
Virofect Enhancer : Please store at - 70º C on arrival
Targefect-MEF : Please store the reagent at - 20º C upon arrival

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Expected efficiency 50-60%. This kit (catalog #MEF-01) contains sufficient reagent for performing 200 transfections in 12-well dishes or 100 transfections in 6-well dishes
Cell types tested: Primary Mouse Embryo fibroblasts, rat fibroblasts, primary human skin fibroblasts, HS-27 cells

Additional instructions for Targefect-MEF

The protocol below using a combination of the Targefect F-2 reagent with Virofect has been reported to work better that the protocol above in some labs for transfection of MEFs so we recommend you test it.

Some additional information on transfection of mouse embryo fibroblasts

We have recent information that suggests that that a combination of the Targefect-F2 reagent and the Virofect enhancer works very well for transfection of mouse embryo fibroblasts. Please try the following two conditions in addition to the transfection protocol that was sent with your product.

Here are the suggested transfection conditions
Cell should be cultured in media with at least 10% serum.
Note : The Targefect F-2 reagent should be stored at 4 °C and gently mixed once on arrival (as the reagent sometimes freezes during shipment. Do not vortex or freeze the Targefect F-2 reagent.

Condition 1 : To 0.5 ml of high glucose DMEM add 12 µg DNA, mix well, Add 12 µl of Targefect F-2, mix well and add 25 µl of Virofect, mix well again and incubate at 37 o C for 25 mins to form transfection complexes. Add 0.5 ml of transfection complex to 2 ml of complete media per 60 mm dish or to 1 ml of complete media per 35 mm dish. Swirl the dish to mix transfection complexes with cell culture media. Incubate overnight and assay at 36-48hrs after transfection.

Condition 2 : To 0.5 ml of high glucose DMEM add 6 µg DNA, mix well, add 12 µl of Targefect F-2 , mix well and add 25 µl of Virofect, mix well again and incubate at 37 °C for 25 mins to form transfection complexes. Add 0.5 ml of transfection complex to 2 ml of complete media per 60 mm dish or to 1 ml of complete media per 35 mm dish. Swirl the dish to mix transfection complexes with cell culture media. Incubate overnight and assay at 36-48hrs after transfeciton**.**
For more information please call Tech support, Targeting Systems, 619-562-1518

Product No.	Quantity	T.S. list Price
MEF-01	1	$250

Contents : Two solutions

Targefect-MEF and Virofect

References citing use of Targefect for transfection of Mouse embryo fibroblasts

1) Nigel Carter, Tetsuya Nakamoto, Hisamaru Hirai, Tony Hunter (2002) Nature Cell Biology4, 565 - 573 (mouse embryo fibroblasts).

2) Robert F. Schwabe and Hiroaki Sakurai(2005) IKK? phosphorylates, p65 at S468 in transactivaton domain 2, FASEB J, 10.1096/fj.05-3736fje.