

Targeting Systems Introduces :

## Targefect-Osteoblast

### For transfection of primary osteoblasts and osteosarcoma cell lines

Transfection of primary rat osteoblasts with the Targefect-Osteoblast transfection kit (transfection efficiency approx 20%). Data courtesy of Dr. TJ Sheu, University of Rochester Medical Center, Rochester, NY.

**Cell types tested Priamry rat osteoblasts, osteosarcoma cell lines U2OS and Saos2, Megakaryocyte cell lines**

### Suggested Transfection protocol

The Targefect-Osteoblast kit is a combination of the Targefect-Osteoblast reagent and the Virofect enhancer for optimal transfection of primary osteoblasts and osteoblastic 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).

Tube #	High Glucose DMEM	DNA	Targefect-Osteoblasts	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. Then add Targefect, mix well again by flicking the tube. Add Virofect last and mix again. Incubate the tubes at 37° C 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- Osteoblast** : Please mix the reagent gently by inverting the tube (do not vortex) and then store at 4 oC upon arrival.

The expected efficiency is 20-40%. This kit (catalog #OST-01) contains sufficient reagent for performing 200 transfections in 12-well dishes or 100 transfections in 6-well dishes.

**Cell types tested: Primary Osteoblasts, Saos2 and U2OS. MEG-1**

Tube #	High Glucose DMEM	DNA	Targefect	Enhancer reagent
1	1 ml	1.5 µg	5 µl F-2	-
2	1 ml	2 µg	5 µl F-1	-
3	1 ml	5 µg	5 µl F-1	5 µl Peptide enhancer
4	1 ml	2 µg	5 µl F-1	5 µl Virofect
5	1 ml	2 µg	6 µl F-2	5 µl Virofect

**Contents : Two solutions**  
**Targefect-Osteoblast and Virofect**