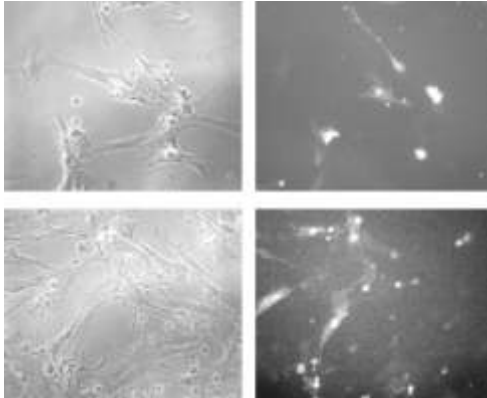


Targeting Systems Introduces :
Targefect-SMC



Primary rat aortic smooth muscle cells (RASM) transfected with an expression vector expressing red fluorescent protein (right panels) using the **Targefect-SMCtransfection kit**:

Cells shown in the LHS panels are phase contrast images of transfected cells shown in the right panel. Data courtesy of Dr Michael Segel, Tufts New England Medical Center, Boston,MA

Transfection protocol

The Targefect-SMC reagent is a combination of the Targefect-SMC reagent with the Virofect enhancer for optimal transfection of primary smooth muscle 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.

Complexing conditions using Targefect-SMC

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

Tube #	DMEM(high glucose)	DNA	Targefect-SMC	Virofect
1	0.5 ml	6 ug	12 ul	-
2	0.5 ml	6 ug	12 ul	25 ul
3	0.5 ml	4 ug	12 ul	25 ul
4	0.5 ml	3 ug	12 ul	25 ul

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.

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 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. Replace the media with fresh complete media the next morning and assay at 36-48 hrs post-transfection.

Virofect enhancer:

Once the optimal ratio of DNA to targetect has been established repeat that experiment titrating the amounts of Virofect enhancer in the transfection complex (enhancer to be added just after the Targetect-SMC reagent).

Expected efficiency - 30-40%

Tested on primary rat aortic smooth muscle cells and primary human aortic smooth muscle cells

This kit (catalog #SMC-01) contains sufficient reagent for performing 200 transfections in 12-well dishes or 100 transfections in 6-well dishes.

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Targetect-SMC Catalogue #	Quantity	Price
SMC-01	1	\$ 250
SMC-10	10	\$ 2,000

Contents: Two solutions

- Targetect-SMC and Virofect (600 ul each)