targetingsystems

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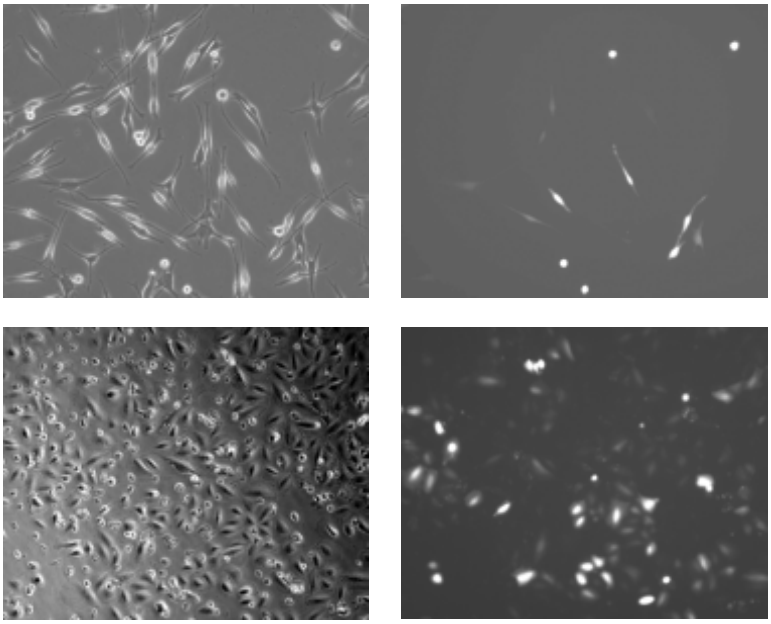
Targeting Systems Introduces :

Targefect-Melanocyte

For transfection of primary melanocytes and melanoma cell lines



Left Panel

Transfection of primary human melanocytes with a GFP expression vector using the Targefect-Melanocyte kit. Lower Panel: Transfection of a melanoma cell line wm793 using the Targefect-melanocyte kit.
Data courtesy of Dr. Karren Adamo and Dr. Andrew Aplin, Albany Medical Center, Albany, New York.

Transfection Protocol

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.
• USE HIGH GLUCOSE DMEM (Dulbecco's modified eagle's medium containing 4500 mg/liter glucose). We recommend trying the following 5 transfection conditions :

Condition 1 :

Complex 3 µg DNA with 6 µl of F-2 and 10 µl of Virofect in 0.25 ml of high glucose DMEM.
Incubate at 37 °C for 25 mins to form transfection complexes. Add 0.25 ml of transfection complex to 2 ml of complete media (DMEM plus 10% FBS) covering cells in 1 well of a 6-well dish. Mix well. After 2 hrs aspirate all the media and replace with 2 ml fresh complete melanocyte media. This condition was used for the transfection of primary human melanocytes shown in the attached picture. Transfection efficiency approx 10%.

Condition 2 :

Complex 3 µg DNA with 6 µl of F-2 and 10 µl of Virofect in 0.25 ml of high glucose DMEM.
Incubate at 37 °C for 25 mins to form transfection complexes. Add 0.25 ml of transfection complex to 2 ml of complete media (melanocyte media plus 10% FBS) covering cells in 1 well of a 6-well dish. Mix well. After 2 hrs aspirate all the media and replace with fresh complete melanocyte media.

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Condition 3 :

Complex 6 µg DNA with 6 µl of F-2 and 10 µl of Virofect in 0.25 ml of high glucose DMEM.
Incubate at 37 °C for 25 mins to form transfection complexes. Add 0.25 ml of transfection complex to 2 ml of complete media (DMEM plus 10% FBS) covering cells in 1 well of a 6-well dish. Mix well. After 2 hrs aspirate all the media and replace with 2 ml fresh complete melanocyte media.

Condition 4 :

Complex 1.5 µg of DNA with 6 µl of F-2 and 10 µl of Virofect 1ml of DMEM
Add 1 ml of transfection media per well of a 6 -well dish In this case the cell culture medium covering the cells should be completely i.e. aspirated before addition of transfection complexes to the cells Incubate cells with transfection complexes at 37 °C overnight and then replace media with complete melanocyte media the next day. Assay at 36-48hrs after transfection. In the experiment whose results are shown in the attached wm793 melanoma pictures the complexes were prepared according to condition 4 Transfection efficiency approx 35%

Condition 5 :

The attached pictures show some toxicity to the melanoma cells. To reduce toxicity we recommend performing a duplicate transfection forming transfection complexes exactly as described in condition 4 but aspirating the complexes after 4 hrs and replacing with 2 ml of complete melanocyte media.

Expected efficiency Approx 10-15 % for primary human melanocytes; 35%-40% for melanoma cell lines.

Product No.	Quantity	T.S. list Price
MEL-001	100 reacns	$250