

Targeting Systems Introduces :

## Targefect-Bronchial

### Suggested transfection protocol for Bronchial epithelial cells

#### Cells incubated with transfection complexes in the absence of serum

Please vortex F-1 reagent at full speed for 30 seconds once or twice just before use. Do not vortex the f-2 reagent or Virofect Conditions recommended most are in bold

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	2 µg	5 µl F-1	15 µl Virofect
4	1 ml	2 µg	6 µl F-2	15 µl Virofect

Add DMEM first, mix well, then add targefect, mix well, Add enhancer last and mix well again. Incubate at 37 °C to form transfection complexes.

Add 1ml of transfection complex per well of a 6-well dish, Add 0.3 ml of transfection complex per well of a 12-well dish or 0.15 ml of transfection complex per well of a 24 well dish (make sure cells are covered well or else add 0.2 ml complex per well. Incubate cells with transfection complexes at 37 °C for 2 hrs. Add complete media with serum, add 0.5 ml complete media per well of a 24 well dish, 1 ml per well of a 12-well dish, 2ml per well of a 6-well dish. Mix gently and incubate overnight. Replace media the next day. Assay at 30-48hrs post transfection. We recommend using media with 10% serum as the complete media.

#### Cells incubated with transfection complexes in the absence of serum

Tube #	High Glucose DMEM	DNA	Targefect	Enhancer reagent
1	0.5 ml	6 µg	12 µl F-1	25 µl Virofect
2	0.5 ml	6 µg	12 µl F-2	25 µl Virofect

Add DMEM first, mix well, then add targefect, mix well, Add enhancer last and mix well again. Incubate at 37 °C to form transfection complexes. Add 70 µl of transfection complex to 0.5 ml of complete media per well of a 24 well dish.

Add 250 µl of transfection complex to 1ml of complete media (i.e. media with 10% serum) per well of a 6- well dish. Swirl the dish to mix transfection complexes with complete media. Assay at 30-48 hrs post transfection

**These last two conditions are highly recommended.**

- Complex 6 µg DNA with 24 µl F-1 and 24 µl of Virofect in 0.6 ml DMEM. Incubate at 37 °C for 25 mins to form complexes. Add 0.2 ml of transfection complex to 2 ml of complete media per well of a 6-well dish immediately after plating cells or add 0.05 ml complex to 0.5 ml of complete media in one well of a 24 well dish.
- Complex 6 µg DNA with 24 µl F-1 and 48 µl of Virofect in 0.6 ml DMEM Incubate at 37 °C for 25 mins to form complexes. Add 0.2 ml of transfection complex to 2 ml of complete media per well of a 6-well dish immediately after plating cells or add 0.05 ml complex to 0.5 ml of complete media in one well of a 24 well dish.

**NOTE: THE CELLS SHOULD BE PLATED IN COMPLETE MEDIA CONTAINING 10% SERUM**

Assay at 36-48 hrs post transfection

These last two conditions give very high efficiencies in other difficult to transfect cells.

## Suggested transfection protocol for Bronchial cells

**Please vortex F-1 reagent at full speed for 30 seconds once or twice just before use. Do not vortex the F-2 reagent or virofect.** Conditions recommended most are in bold.

### PROTOCOL 1

**Transfection of cells in presence of serum :**

**Step1 :** Preparation of transfection complexes -

Please prepare transfection complexes using serum free DMEM. Use 2 ml or 5 ml sterile clear polystyrene tubes preparing transfection complexes. Make additions as follows:

Tube #	High Glucose DMEM	DNA	Targefect	Enhancer reagent
1	0.5 ml	6 µg	12 µl F-1	25 µl Virofect
2	0.5 ml	6 µg	12 µl F-2	25 µl Virofect
3	0.5 ml	12 µg	12 µl F-2	25 µl Virofect
4	0.5 ml	6 µg	12 µl F-1	
5	0.5 ml	10 µg	20 µl F-2	

Add DMEM first, mix well, then add targefect, mix well, Add enhancer last and mix well again . Incubate at 37 oC for 25 mins to form transfection complexes.

Add 250 µl of transfection complex to 2 ml of complete media (with 10 % serum) per well of a 6 well dish.

Add 125 µl of transfection complex to 0.5 ml of complete media (with 10% serum) per well of a 12 well dish.

Add 70 µl of transfection complex to 0.5 ml of complete media per well of a 24 well dish.

Swirl the dish to mix transfection complexes with complete media. Replace media with fresh culture media the next day. Assay at 30-48 hrs post transfection.