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Virofect

Virofect is an adenovirus-derived enhancer formulation that significantly enhances the efficiency of gene delivery and transgene expression in a wide variety of cell types when used in combination with the Targefect F-1 or F-2 reagents. Virofect also enhances the efficiency of gene transfer when used in combination with other cationic reagents (see references 1, 2) such as lipofectin, lipofectamine and Superfect. Virofect enhances gene transfer by using adenoviral receptors on the cell surface to enhance intracellular delivery of transfection complexes. Once the transfection complex is internalized, Virofect helps the transfection complex escape degradation in the lysosome and enhances the duration of transgene expression

Cell seeding: Set up cells so that they are approx 70% confluent at the time of the experiment

Preparation of the complexes and transfection procedure:

Gently thaw the Virofect enhancer just before use. The Virofect enhancer is used in combination with the Targefect F-2 or the Targefect F-1 reagents. Since reagents sometimes freeze during shipping, we recommend gently mixing the Targefect-F2 solution once upon receipt. The Targefect-F-2 reagent should be stored at 4°C. Do not vortex the Targefect-F2 reagent. The Targefect F-1 reagent which is stored at -20 °C should be thawed and vortexed at full speed for 30 second twice just before use. The Virofect Enhancer should be stored at -20°C or -70°C.

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).

Tube #	High glucose DMEM (serum free)	DNA	Targefect	Enhancer Reagent
1	0.5 ml	6 µg	12 µl F-2	24 µl **Virofect**
2	0.5 ml	6 µg	24 µl Targefect F-1	24 µl Virofect
3	0.5 ml	6 µg	12 µl Targefect F-1	24 µl of Virofect

Add DMEM first. 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. Incubate the tubes at 37°C for 25 minutes to form the transfection complexes.

Condition above is for transfecting cells in the presence of serum according to our fast protocol: 250 µl of transfection complex is added to 1 ml of complete media (with serum) per well of a six-well dish. The dish is swirled to enable mixing of the transfection complex with the cell culture medium and the cells are incubated at 37 °C overnight and assayed for gene expression 36-48 hrs post transfection. Add 0.5 ml of transfection complex to 2 ml of complete media with serum for one 60 mm dish. For transfecting cells in a 6-well dish add 0.25 ml of transfection complex to 1 ml of complete media, for a 12-well dish add 0.125 ml transfection complex to 0.5 ml of complete media. Swirl the dish to gently mix transfection complexes with the cell culture media. Incubate overnight. Assay at 24-48 hrs after transfection.

Note: in some cases such as transfection of Min cells and other pancreatic cell lines using a combination of the Targefect F-1 reagent with Virofect, better transfection efficiencies have been achieved by adding transfection complexes to the cells at the time of plating.

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Transfection efficiencies achieved using the Targefect in combination with Virofect

Cell types	Transfection efficiency
HUVECs (human umbilical vein endothelial cells) **(F-2 plus Virofect)**	70%-90%
Human dermal microvascular endothelial cells **(F-2 plus Virofect)**	30-40%
Human lung microvascular endothelial cells **(F-2 plus Virofect)** Human aortic endothelial cells	70%-90%
(F-2 plus Virofect) Bovine aortic endothelial cells	50%
(F-2 plus Virofect)	60%
Porcine endothelial cells	60%
Rat endothelial cells **(F-2 plus Virofect)**	60%
Various endothelial cell lines INS-1 and Min 6 pancreatic cell lines **(F-1 plus Virofect)** MCF-7 (F-2 plus Virofect)	40-90% 60-80% 60%
Rat and mouse , Hepatocytes , human Hepatocytes (Targefect **F-1 plus Virofect**)	50-80%
Targefect-Chondrocyte, Targefect-osteoblast, Targefect-BAC Targefect-Hepatocyte, Targefect-Melanoma, Targefect-PCL Targefect-Raw all contain Virofect as a kit component.	Human Hepatocytes give lower efficiency appropx 10%
Targefect-Chondrocyte, Targefect-osteoblast, Targefect-BAC Targefect-Hepatocyte, Targefect-Melanoma, Targefect-PCL Targefect-Raw all contain Virofect as a kit component.	

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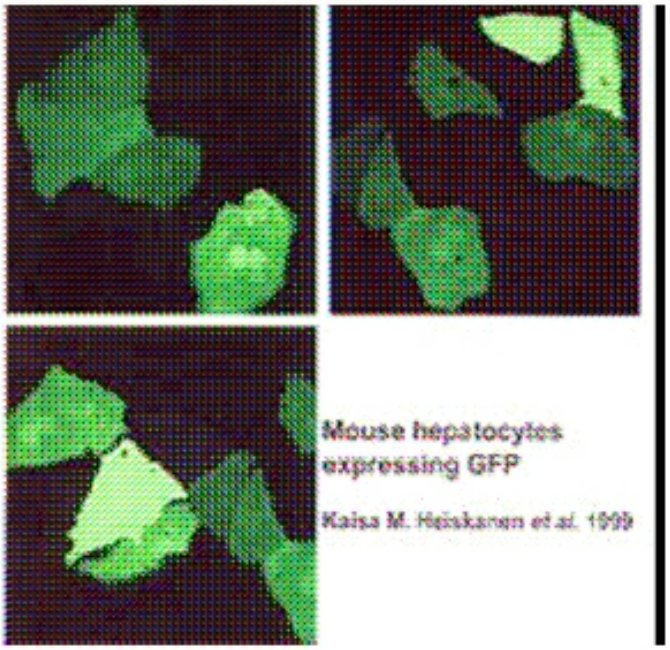
Precautions and handling: Virofect contains a replication-deficient Adenovirus preparation. Because of the nature of this component, it should not be used with cell lines that contain Adenovirus sequences such as HEK-293, to avoid complementation of the virus. Additionally, it is recommended that common laboratory bio-safety used in standard Adenovirus work should be practiced. For more information see http://bmbl.od.nih.gov/sect3bsl2.htm

Panel A



Panel B

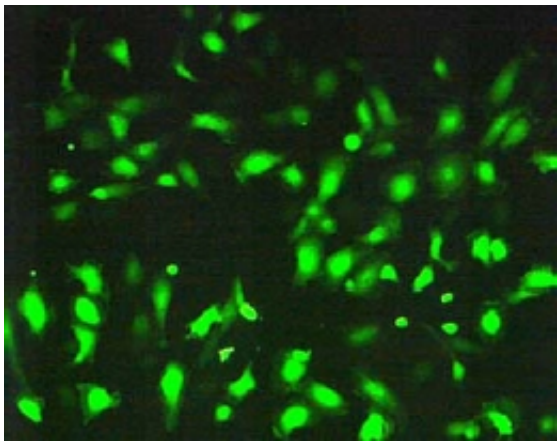


Figure 1A: Mouse hepatocytes transfected with **Targefect- F1 plus Virofect enhancer**
Data courtesy of Dr K. Haiskanen and Dr A. Nieminen, Case Western Reserve Univ., Cleveland OH
Figure 1B: Human umbilical vein endothelial cells transfected with the Targefect F-2 reagent plus Virofect enhancer. (for details refer to the Targefect-HUVEC product brochure on our website)
Data courtesy of Dr Michael Potente. University of Frankfurt, Germany.

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Storage Conditions

Storage Temperature: -20°C

Technical Support
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