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Peptide Enhancer

The peptide enhancer is a modified protein based formulation which enhances gene transfer by increasing transgene delivery to the nucleus when used in combination with the Targefect F-1 or F-2 reagents. Once the transfection complex is internalized, the peptide enhancer helps the transfection complex escape degradation in the lysosome and enhances the duration of transgene expression.

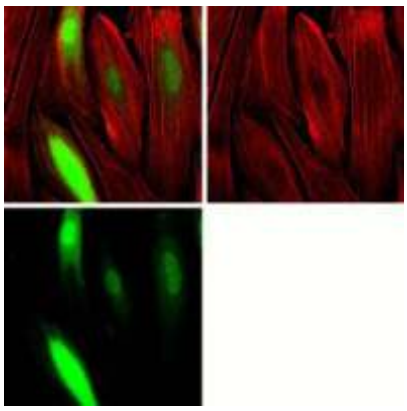

Figure 2:
Transfection of micro vascular endothelial cells (human) with a green fluorescent protein expression vector using **Targefect-F2** and the **Peptide enhancer**: Confocal images of cells transfected with a GFP-expression vector and counter-stained with rhodamine-phalloidin (actin stain) (Data courtesy of Dr. Steve Duffy and J. Murphy, UT, Southwestern Medical Ctr., Dallas, TX)

Transfection Protocol:

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 mix the peptide enhancer just before use. The peptide 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 Peptide Enhancer should be stored at 4oC.

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 #	DMEM(high glucose)	DNA	Targefect	Peptide Enhancer
1	0.5 ml	6 µg	12 µl F-2	24 µl Peptide Enhancer
2	0.5 ml	0.6 µg	10 µl F-2	20 µl of Peptide Enhancer
3	0.5 ml	6 µg	24 µl Targefect F-1	24 µl of Peptide Enhancer
4	0.5 ml	6 µg	12 µl Targefect F-1	24 µl of Peptide Enhancer

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. Next add Peptide Enhancer, Mix again by flicking the tube. Incubate the tubes at 37°C for 25 minutes to form the transfection complexes.

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Conditions above are for transfecting cells in the absence of serum:

Aspirate off cell culture media just before addition of transfection complexes. 250 µl of transfection complex is added to 0.6 ml of DMEM and then added to each well of a six-well dish. The dish is swirled and incubated for 3 hrs at 37°C. At the end of 3 hrs 2 ml of complete media (with serum) is added to each well and the dish is swirled again 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 36-46 hrs post transfection. The conditions described are for a 35 mm dish or one well of a 6-well dish. The volume of transfection complex added to each well depends on the size of the well or dish and should be sufficient to cover cells well (without drying) for a 3 hr period.

References

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2. Smarajit Bandyopadhyay, Mohammad Z. Ashraf, Pamela Daher, Philip H. Howe, and Paul E. DiCorleto (2007) HOXA9 Participates in the Transcriptional Activation of E-Selectin in Endothelial Cells Mol. Cell. Biol., Jun 2007; 27: 4207 - 4216.

3. Harinivas H. Krishnan, Neelam Sharma-Walia, Ling Zeng, Shou-Jiang Gao, and Bala Chandran (2005) Envelope Glycoprotein gB of Kaposi's Sarcoma-Associated Herpesvirus Is Essential for Egress from Infected Cells J. Virol., Sep 2005; 79: 10952 - 10967.

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Storage Conditions

Storage Temperature: 4°C

Technical Support

For questions regarding this product please contact our tech support at 1-619-562-1518 or email us targsys@aol.com

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