PepFect

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TRANSFECTION PROTOCOL

Description

PepFect is a patented proprietary transfection agent based on a formulation of a chemically modified cell-penetrating peptide specifically developed for condensing oligonucleotides into nanoparticles for intracellular delivery.

Unlike many other transfection agents, PepFect can transfect nearly 100% of the cell population and efficiently release the complexed oligonucleotides from endosomes, rendering the delivered oligonucleotides active at their respective targets. Chemical modifications in PepFect and its buffer composition ensure that the formulation is stable and active in various cell culture conditions[1].

For example results and tested cell types, please consult the reference.

Preparation of PepFect stock solution

Prepare PepFect transfection reagent 100x stock solution as described below:

1. Reconstitute one vial of PepFect in 500 µl Reconstitution Buffer (1 mM TFA).
   Note: This yields a 100x stock solution.

2. Mix and vortex the vial with reconstituted PepFect to ensure that the peptide is entirely dissolved.

3. Store the dry PepFect and its stock solution at –20°C.

Materials required but not provided with the PepFect transfection reagent:

- OptiMEM medium (Life Technologies)
- 1 mM trifluoroacetic acid (TFA) in water

Reference

1. PepFect14, a novel cell-penetrating peptide for oligonucleotide delivery in solution and as solid formulation, *Nucleic Acids Res.* 2011, 39(12), 5284-98.

Transfection procedure

These instructions are for transfecting cells that are seeded in 24-well tissue culture plates (surface area 2 cm^2).
For transfections in tissue culture plates with a surface area other than 2 cm^2, please adapt volumes and amounts accordingly.

For transfecting 1 well of a 24-well tissue culture plate do the following:

Day 0	Seed cells into a 24-well tissue culture plate in 450 µl normal growth medium per well.
Day 1	Note: Prepare PepFect complexes in 1/10th of the final transfection volume. The final transfection volume after adding the complexes will be 500 µl per well.
	1. In volume of 50 µl OptiMEM, mix 50 pmol antisense oligonucleotide (AON) or 10 pmol siRNA or 0.25 µg plasmid DNA with 0.5 µl reconstituted 100x stock solution of Pepfect.
	2. Incubate the mixture at room temperature for 30 min to allow complex formation.
	3. Add 50 µl of the formed PepFect complexes to cells grown in 450 µl of normal growth media. Note: the final concentration of the added oligonucleotides will be 100 nM AON, 20 nM siRNA and 0.5 µg/ml plasmid DNA.
	4. After 4 hours, remove the added complexes and replace with fresh cell growth media.
Day 2	24 hours after adding the complexes, measure the activity of the transfected AON, siRNA or plasmid.

Note: The amount of antisense oligonucleotide, siRNA and plasmid DNA can be varied to optimise the transfection conditions, depending on application.

Scaling

The amount of PepFect to be used per one well in different types of plates.

	Approximate surface area (cm^2)	Scaling factor	PepFect 100x stock solution
96-well plate	0.32	0.16	0.08 µl
48-well plate	0.95	0.48	0.24 µl
24-well plate	2.0	1.0	0.5 µl
12-well plate	3.8	1.9	1.0 µl
6-well plate	9.5	4.8	2.4 µl

Example results