

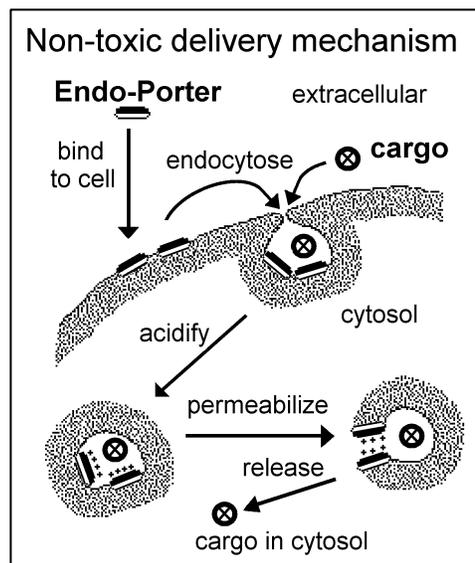
ENDO-PORTER DELIVERY OF MORPHOLINO OLIGOS

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I. INTRODUCTION

Endo-Porter is a reagent for delivering Morpholino oligos, peptides or proteins into the cytosol of cultured cells. Endo-Porter is a peptide-based reagent which enters cells by endocytosis, works in the presence of 10% serum, and allows delivery of multiple oligos at a range of oligo concentrations.

Delivery is least toxic when the process does not disrupt the plasma membrane. The mechanism of Endo-Porter delivery relies on Morpholinos and Endo-Porter being taken up from the media into the same endosome. Endo-Porter is an amphiphilic peptide with a sharp transition pH. It has a hydrophobic face which associates with cell membranes once added to culture medium. Morpholinos or other cargo molecules in the medium may be co-endocytosed with Endo-Porter. Natural acidification of the endosome protonates Endo-Porter and then, in its ionic form, Endo-Porter permeabilizes the endosome and releases the endosome contents into the cytosol (Summerton, 2005).



Gene Tools provides Endo-Porter in three formulations, an aqueous PEG formulation, a DMSO formulation, and an aqueous formulation with mannitol. The aqueous PEG formulation contains 1mM Endo-Porter peptide and 10% polyethylene glycol 1500 mw in water. The aqueous PEG formulation combines the advantages of the older formulations and is our only recommended form of Endo-Porter, but we continue to offer the Endo-Porter DMSO and older Endo-Porter Aqueous formulations to service legacy projects.

MSDS are posted at: www.gene-tools.com/MSDS

II. DELIVERING MORPHOLINO OLIGOS with Endo-Porter

A. Preparation of Reagents

Morpholino Stock Solutions

Custom-Sequence Oligo: To one vial containing 300 nanomoles of sterile freeze-dried custom-sequence Morpholino oligo add 0.3 ml of sterile water to give 0.3 ml of a 1 mM oligo stock solution.

Standard Control Oligo: To one vial containing 100 nanomoles of sterile freeze-dried Standard Control Morpholino oligo add 0.1 ml of sterile water to give 0.1 ml of a 1 mM oligo stock solution.

Endo-Porter Delivery Reagent

Endo-Porter PEG is provided as a 1.0 mM sterile solution prepared in 10% polyethylene glycol 1500 mw in water. (The DMSO formulation is Endo-Porter peptide in neat DMSO and the Endo-Porter Aqueous formulation is Endo-Porter peptide in 0.3 M mannitol solution.) Endo-Porter can be stored at room temperature or refrigerated. It should be warmed to room temperature before use but requires no further preparation.

B. Preparing Cells for Endo-Porter Delivery

Cells can be cultured in plates or flasks and for best delivery should be 80-100% confluent.

C. Protocol for Delivery of Morpholinos with Endo-Porter

1. Replace spent culture medium with fresh complete medium (with up to 10% serum).
2. Add Morpholino stock solution to the desired concentration and swirl well to mix.

Morpholinos are typically effective at concentrations as low as 1 μM (1 μl of 1 mM stock per ml of media). For initial experiments, especially if you are evaluating Morpholino delivery via fluorescence, we recommend starting with 10 μM Morpholino oligo concentration (10 μl of 1 mM stock per ml of media) and then reducing or increasing your Morpholino concentration based on your results.

3. Add 6 μl of Endo-Porter for every 1 ml media and immediately swirl to mix. This provides a final Endo-Porter concentration of 6 μM .
4. Once all samples have been treated return plates/flasks to the incubator.

Cells can be assayed as soon as 16 hours after treatment and Endo-Porter can be left in the medium up to 72 hours without damage to your cells. We have found minimal toxicity and maximal delivery by assaying 48 hours after delivery (see figure below). In the HeLa positive antisense test system that generated these results, delivering the Standard Control Morpholino (3 μM) corrects a splicing error and puts luciferase in frame (Kang et al. 1998).

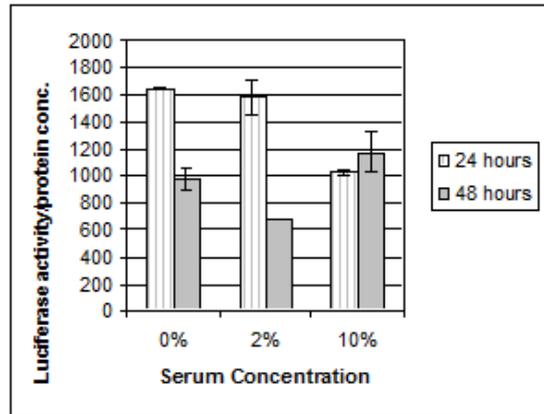
D. Storage of Reagents

Morpholino stock solutions should be stored at room temperature in the original vial and preferably at 1 mM. Endo-Porter is a stable peptide and can be stored in solution at room temperature for many months.

E. Frequently Asked Questions

1) What is the effect of serum on Endo-Porter delivery?

Endo-Porter is unique among delivery reagents as it works in complete (up to 10%) serum-containing media with only a small decrease in delivery efficacy. As shown below, the longer the Endo-Porter delivery period the smaller the difference between using serum-free and 10% serum-containing media. The data in the figure were generated from delivering 3 μM Morpholino to 80% confluent HeLa positive antisense test system (Kang, 1998).



2) How do I scale the volume of Endo-Porter for different size plates and flasks?

Endo-Porter delivery of Morpholinos is a concentration-dependent process and by keeping concentration constant you should expect no differences in delivery when using a lesser or greater volume of media.

3) Is Endo-Porter toxic?

Endo-Porter can NOT be washed off cells. Toxicity from Endo-Porter depends on the time and concentration that the cells are exposed to the Endo-Porter peptide. We have found minimal toxicity and maximum delivery using the recommended Endo-Porter concentration (6 μM) and assaying antisense activity at 48 hours.

4) May I optimize Endo-Porter delivery for my cell type?

Yes, in an initial study that follows your research protocol we recommend delivering your cargo (Morpholino, protein, peptide, etc.) using Endo-Porter in a range of concentrations from 2 to 10 μM (from 2-10 μl per ml of media) increasing in 2 μM increments and observing your cells for toxicity and delivery efficacy. We recommend you find the concentration that provides you with maximal delivery and minimal toxicity to use for further experiments.

5) How do I deliver proteins and peptides with Endo-Porter?

For proteins and peptides add the desired concentration to the medium, add 6 μl of Endo-Porter per ml of media and swirl to mix.

6) Which formulation of Endo-Porter should I purchase?

We recommend purchasing the Endo-Porter PEG formulation. Because cultured cells can tolerate higher doses of this formulation (up to 20 $\mu\text{L}/\text{ml}$), higher efficacies can be achieved compared to the less-effective formulations, Endo-Porter DMSO and Endo-Porter Aqueous.

7) Does Endo-Porter deliver Morpholinos in vivo?

Endo-Porter PEG can be used for central nervous system (CNS) injections. Choose the high concentration (4mM) Endo-Porter PEG for CNS applications.

Endo-Porter was specifically developed for in vitro applications. It may be effective in some in vivo systems where both Morpholino and Endo-Porter can be co-endocytosed into the same endosome. Gene Tools is working on the development of methods for functional in vivo delivery of Morpholinos. New delivery products will be announced on www.gene-tools.com when they become available.

8) What procedures should I use to assess delivery by fluorescence?

When using Endo-Porter to deliver a fluorescent molecule, delivery may be assessed via flow cytometry or visually using an inverted epifluorescent microscope. Endo-Porter can deliver enough Morpholino to the cell for antisense activity without depositing perceptible fluorescence in the cells, but during proof-of-delivery experiments we recommend using 10 μ M labeled Morpholino to see the fluorescence.

We recommend you look at live cells as the procedures involved in fixing cells may lead to leakage of Morpholinos into the cells and a false positive signal. Observe the cells periodically so you see changes in the pattern of the fluorescence over time. If you see diffuse fluorescence throughout the cytosol of the cells, you have delivered the Morpholino oligos. Using higher numerical aperture objective lenses (higher magnification objectives) can help increase the amount of light gathered from a cell and reveal dim fluorescence.

9) What cell types can Endo-Porter deliver?

We expect Endo-Porter to deliver cargo to any cell type that undergoes endocytosis followed by acidification of the endosome.

F. References

Kang SH, Cho MJ, Kole R. (1998) Up-regulation of luciferase gene expression with antisense oligonucleotides: implications and applications in functional assay development. *Biochemistry*. 37(18):6235-39.

Summerton JE. Endo-porter: a novel reagent for safe, effective delivery of substances into cells. *Ann N Y Acad Sci*. 2005 Nov;1058:62-75.