

Assaying Delivery of Morpholinos into the Cytosol Using Fluoresceinated Oligos

To check cytosolic delivery with a fluoresceinated oligo, follow the delivery protocol shipped with the delivery reagent or system. Endo-Porter is the preferred delivery reagent because of its simplicity, effectiveness and lack of toxicity. If you are using Endo-Porter, it is best to deliver single-stranded Morpholino oligos. For the Special Delivery system, use a Special Delivery oligo (Morpholino/DNA heteroduplex) and the provided EPEI delivery reagent.

Look at live cells. Do not fix the cells as this can cause leakage of Morpholinos from the endosome/lysosome compartment into the cytosol of the cells, falsely suggesting cytosolic delivery ⁽²⁾. Observe the cells periodically so you see changes in the pattern of the fluorescence over time. Ideally, observe cells using an inverted epifluorescence microscope fitted with a fluorescein filter cube and, to gather as much light as possible, with a high numerical aperture objective lens. Carboxyfluorescein has an excitation of 495 nm and an emission of 517 nm in aqueous solutions of pH 5 to pH 9 ⁽¹⁾. Carboxyfluorescein can photobleach, so keep labeled oligos in the dark when not in use. When using a fluorescent Morpholino to confirm delivery by Endo-Porter, start with 10 microMolar Morpholino oligo in the culture medium. At this concentration the fluorescent-tagged Morpholino should be visible in the cytosol after 16 hours.

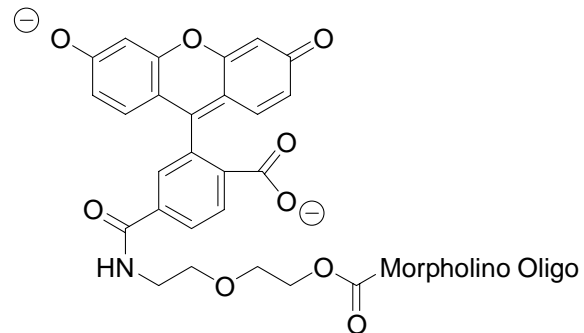
If you see dim fluorescence diffusely spread throughout the cytosol/nuclear compartment of the cell then that indicates successful delivery into the cytosol. If you see punctate fluorescence, this does not indicate delivery to the cytosol (though it does not preclude it). Punctate fluorescence is usually caused by fluorescent oligos trapped inside endosomes, and in these locations a Morpholino will not interact with its target mRNA. Diffuse fluorescence indicating successful delivery to the nuclear/cytosolic compartment may or may not co-exist with punctate fluorescence; ignore the punctate spots. Visible diffuse fluorescence requires a fluoresceinated oligo concentration up to ten times higher than the concentration needed for antisense action against most targets. When you have confirmed delivery by observing diffuse fluorescence then there is more labeled Morpholino in the cytosol than is needed to inhibit translation of most mRNA targets, so the Morpholino can usually be used at lower concentration for subsequent knockdown experiments. Note that flow cytometers or FACS machines cannot distinguish between punctate and diffuse fluorescence so they cannot confirm delivery into the proper subcellular compartment.

Our fluoresceinated oligos use a carboxyfluorescein covalently attached to the oligo through a spacer (a "leash"). The flexible leash is sufficiently long so that the fluor does not interfere with hybridization of the Morpholino to a complementary RNA.

3'-Carboxyfluorescein

Green-emitting fluorescent tag
Mass of end modification: 490 Daltons
End of production number contains -F
Example: 12-04Apr05A-F

Excitation peak: 501.5 nm
Emission peak: 524.5 nm

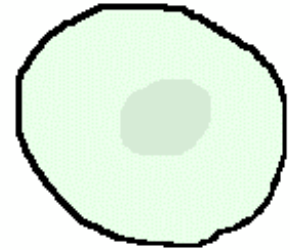


(1) Richard P. Haugland. Molecular Probes Handbook of Fluorescent Probes and Research Chemicals, 7th Edition. 1999. Molecular Probes, Eugene, Oregon, USA.

(2) Moulton HM, Moulton JD. Peptide-assisted delivery of steric-blocking antisense oligomers. *Curr Opin Mol Ther.* 2003 Apr;5(2):123-32.

Interpreting common patterns of fluorescence distribution

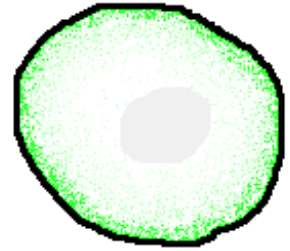
Successful delivery is indicated by dim and diffuse fluorescence throughout the cytosol/nuclear compartment.



Punctate fluorescence indicates at least some oligo is trapped in endosomes. Such trapped oligos cannot interact with pre-mRNA or RNA, and so cannot block splicing or translation.



Unmodified Morpholinos have little interaction with the cell membrane, but some modifications (such as polyamines) make Morpholinos stick to the outside of cells. These cells appear to be outlined with fluorescence.



It is not unusual to see both dim diffuse and brighter punctate fluorescence. The diffuse component indicates successful delivery.

