CellBio> Vol.1 No.2, December 2012

OPEN ACCESS

Optimized Conditions for the Delivery of Small Membrane Impermeable Compounds into Human Cells Using Hypotonic Shift

PDF (Size:441KB) PP. 38-42 DOI: 10.4236/cellbio.2012.12005

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ABSTRACT

Cell-based assays represent a major end point of high throughput screening (HTS) but a key limitation of such assays is the potentially poor membrane permeability of test compounds. In this study, we optimized the conditions for the delivery of the membrane impermeable compound 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) into human cells using hypotonic shift; a method that can promote the uptake of molecules from the extracellular fluid into cell cytoplasm via endocytosis. We showed that uptake of HPTS by cells was a function of hypotonic buffer osmolarity and that delivery was highly efficient with almost 100% of cells displaying uptake. Delivery of HPTS was equally effective at 25°C and 37°C, with delivery of compound proportional to incubation time and concentration of HPTS within the loading medium. The experimental conditions identified in this study could be applied to HTS drug discovery studies providing an effective method of delivering small membrane impermeable compounds into cells.

KEYWORDS

Hypotonic Shift; Cell-Based Assay; Membrane Impermeable Compound Delivery; Osmolarity; Endocytosis

Cite this paper

A. Stephens, C. Day and J. Tiralongo, "Optimized Conditions for the Delivery of Small Membrane Impermeable Compounds into Human Cells Using Hypotonic Shift," *CellBio*, Vol. 1 No. 2, 2012, pp. 38-42. doi: 10.4236/cellbio.2012.12005.

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