PowerLoad™ Concentrate

Table 1. Contents and storage information.

Product	Amount	Concentration	Storage	Stability
PowerLoad™ Concentrate	5 mL	100X solution in sterile water	• 2–6˚C • DO NOT FREEZE	6 months

Introduction

PowerLoad™ concentrate is an optimized formulation of nonionic, Pluronic® surfactant polyols designed to aid the solubilization of water-insoluble dyes and other materials in physiological media.[1] Surfactant reagents such as Pluronic® F-127, have been used to help disperse acetoxymethyl (AM) esters of fluorescent ion indicators such as fluo-4, fura-2, indo-1, fluo-3, and SBFI; they appear to be required for loading of other dyes (e.g., SBFI-AM or PBFI-AM). The use of PowerLoad™ concentrate is optional with large molecular weight AM ester, cell-permeable dyes, and may also be useful for dispersing other lipophilic probes. The working concentration of Pluronic® surfactants in PowerLoad™ concentrate is less than 0.2%. PowerLoad™ concentrate is effective in combination with water soluble Probenecid (Invitrogen Cat. no. P36400) to aid AM ester dye-loading and retention in cells that actively extrude the de-acetylated form through anion pumps. Together, these reagents allow for maximal loading of dyes with minimal effort in imaging and high throughput screening (HTS) applications. Appropriate controls should be performed to make certain that PowerLoad™ concentrate is not altering the membrane properties of the cell.

Guidelines for Use

Using PowerLoad™ Concentrate

The experimental conditions for loading cells with AM esters varies with cell type due to differences in probe uptake, anionic pump activity, and in the intracellular esterase activity required for hydrolysis of the AM esters. Keep solutions of the AM esters in anhydrous DMSO since the solvent readily takes up moisture, leading to AM ester hydrolysis and loss of cell-loading efficacy. Add PowerLoad™ concentrate only to working solutions. Typically, a small volume of the AM ester, dissolved at 1–10 mM in DMSO, is premixed into 100X PowerLoad™ concentrate immediately before dissolving to the final volume in aqueous loading buffer. This solution of AM ester and PowerLoad™ concentrate is then diluted into the cell-loading buffer to achieve a final AM ester concentration of between 1–20 µM and the cells are incubated for between 10 minutes and 1 hour at temperatures ranging from room temperature to 37˚C. More weakly fluorescent indicators, such as the

AM esters of SBFI, PBFI, quin-2, and Fura Red™ may require more concentrated loading solutions and correspondingly greater amounts of dye in the stock solution.[2] In general it is desirable to use the minimum amount of AM ester needed to achieve adequate fluorescence signal to noise levels. Loading may be done at any temperature that is tolerable for the cells. Note that the incubation temperature generally affects the extent of intracellular dye compartmentalization.[3,4] When used together with water soluble Probenecid the dye retention is maximized in cells that actively extrude dye and lose signal over time. After labeling, the cells are washed with **fresh medium** (containing water soluble Probenecid) before beginning the experiment.

Sample Protocol

A sample protocol for using PowerLoad™ concentrate with fluo-4 or red-shifted calcium indicators is described below.

Prepare 10 mL loading buffer as follows:

1.1 Prepare 5 mM AM ester stock of the indicator dye in anhydrous DMSO. Store any unused portions of the dye at –20˚C, protected from light and moisture. Avoid repeated freeze-thaw cycles.

1.2 Transfer 100 µL of PowerLoad™ concentrate into a 15 mL conical tube. Add 10 µL of 5 mM dye in DMSO and swirl vigorously to completely dissolve the dye stock.

1.3 Add 10 mL of buffered saline or the desired loading buffer to the tube to obtain a final 1X (<0.2%) concentration of PowerLoad™ concentrate and 5 µM AM ester of the dye. Mix well.

Note: If desired, separately prepare 100X water soluble Probenecid by adding 1 mL of aqueous solution to a single 77 mg vial of water-soluble Probenecid (Invitrogen Cat. no. P36400), and add 100 µL of Probenecid solution to 10 mL loading buffer to further enhance the loading of the dye by blocking anion pumps that extrude the de-esterified dye form. Store any unused aliquots of Probenecid solution at –20˚C to avoid freeze-thaw cycles.

1.4 Remove the culture medium from cells and immediately replace with loading buffer (prepared in step 1.3). A typical starting point for loading time and temperature is 30 minutes at 37˚C (atmospheric CO_2) followed by 30 minutes at room temperature. However, many dyes load effectively with single temperature/time protocols, and compartmentalization of some dyes may be enhanced at warmer temperatures. Optimization of loading time and temperature for each combination of cells and dye used maybe necessary to insure the most consistent results.

1.5 After loading is complete, remove the loading buffer and immediately replace with buffer containing water soluble Probenecid but without dye and PowerLoad™ concentrate to keep the dye trapped in cells for imaging or HTS analysis.

References

1. J Membrane Biol 19, 1 (1974); **2.** J Biol Chem 265, 19543 (1990); **3.** Methods Enzymol 302, 341 (1999); **4.** Methods Enzymol 307, 441 (1999).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
P10020	PowerLoad™ Concentrate, 100X	5 mL
Related Products		
P36400	Probenecid, water soluble	10 × 77 mg
P6866	Pluronic® F-127 *10% solution in water* *0.2 µm filtered*	30 mL
P6867	Pluronic® F-127 *low UV absorbance*	2 g
P3000MP	Pluronic® F-127 *20% solution in DMSO*	1 mL

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