

## Probenecid, water soluble

**Table 1.** Contents and storage information.

Material	Amount	Storage	Stability
Probenecid, water soluble	10 × 77 mg	<ul style="list-style-type: none"> <li>• ≤25°C</li> <li>• Desiccate</li> </ul>	When stored as directed the product is stable for 6 months

### Introduction

Probenecid is commonly used to inhibit organic-anion transporters located in the cell membrane. Such transporters can extrude dyes and indicators and thus contribute to poor loading or a high background signal in assays based on retention of the dyes or indicators inside cells. For example, fluo-4, AM is a Ca<sup>2+</sup> indicator widely used for in-cell measurement of agonist-stimulated and antagonist-inhibited calcium signaling. The acetoxymethyl ester (AM) dye precursor is uncharged, cell permeant, and nonfluorescent. Inside the cell, nonspecific esterases cleave the AM blocking groups, generating the charged, active form of fluo-4 which fluoresces upon Ca<sup>2+</sup> binding. Although the charged dye molecules leak out of cells far more slowly than the uncharged precursor molecules, the anion transporters promote the leakage of the fluorescent dye over time. The use of probenecid to block the efflux of intracellular dyes was first reported by Di Virgilio et al.<sup>1</sup>

Wash steps or quencher dyes may be incorporated into fluorescent assays to minimize baseline fluorescence. However, washing introduces an extra step that is undesirable for high-throughput applications, and may also risk loss of non-adherent cells. Quencher dyes, while offering the advantage of homogeneous (one-step, mix-and-read) assays, may interact negatively with some receptor systems of interest.<sup>2</sup> The use of probenecid to suppress efflux of fluorescent dyes is a favorable method for reducing baseline fluorescence. Our water-soluble probenecid, available as a stand-alone product or supplied with our Fluo-4 NW (no-wash) Calcium Assay Kits, has the advantages of being easy to dissolve in buffer and safer to use than the commonly used free acid form, which requires caustic 1 M NaOH to dissolve it. It is also slightly more effective than the free acid form at equimolar concentrations, probably due to its better solubility (Figure 1).

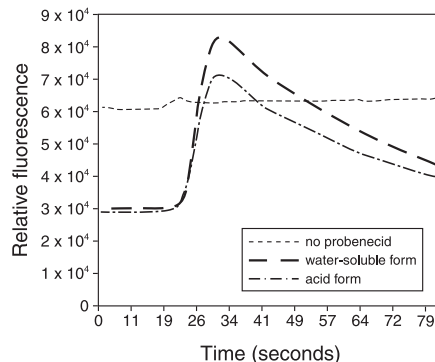
### Guidelines for Use

#### Using Probenecid

Store the dry, solid probenecid desiccated at ≤25°C. Dissolve the contents of one vial in 1 mL of buffer, such as HBSS, and use the solution the same day or store it at ≤-20°C for up to 6 months. This stock solution of probenecid is at a concentration of 250 mM. Typical working concentrations in cell-based assays are 1–2.5 mM.

## References

1. Cell Calcium 11, 57 (1990). 2. Biotechniques 34, 164 (2003).



**Figure 1.** Inhibition of dye extrusion using 2.5 mM probenecid. CHO M1 cells were incubated in dye loading solution for 60 minutes at 37°C, which accentuates the probenecid effect. Calcium release was triggered in CHO M1 cells (M1WT3 cell line, ATCC #CRL-1985) using 20 nM carbachol. The averages of four measurements (without baseline subtraction) in a Fluo-4 NW assay were plotted.

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

Cat. no.	Product Name	Unit Size
P36400	Probenecid, water soluble .....	10 x 77 mg
P10020	PowerLoad™ Concentrate, 100X .....	5 mL
F36205	Fluo-4 NW Calcium Assay Kit (high-throughput) *for 100 microplates* .....	1 kit
F36206	Fluo-4 NW Calcium Assay Kit (starter pack with buffer) *for 10 microplates* .....	1 kit

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