ReadiUseTM Probenecid *25 mM Stabilized Aqueous Solution*

Ordering Information	Storage Conditions
Product Number: 20062 (10x10 mL, 10x10	Expiration date is 6 months from the date of receipt
plates)	Store at -20 ^o C, protected from light

Introduction

Probenecid is an inhibitor of organic-anion transporters located in cell membranes. These transporters often extrude fluorescent indicators from cells, and thereby contribute to poor dye retention. This phenomenon usually causes high background in the assays that require the good retention of the dye indicators inside cells. The use of probenecid to inhibit the activity of the transporter, and thus to reduce leakage of the intracellular dye indicators is a common method for reducing fluorescence background of calcium assays. The commonly used free acid form of probenecid requires the use of 1 M NaOH to dissolve it due to its poor water solubility in neutral water. Our ReadiUseTM probenecid 25 mM stabilized aqueous solution containing 0.2% Pluronic® F-127 is convenient to use and as effective as the free acid form at the same concentration. It can be used to prevent florescent dyes (such as Indo-1 AM, Fura-2 AM, Fluo-3 AM, Fluo-4 AM, Fluo-8 AM, Rhod-2 AM and Rhod-4 AM) from leaking out of cells.

Protocol (for 1 plate)

- 1 Thaw 1 bottle of Cat. # 20062 at room temperature before use.
- 2 <u>Make 2.5 mM ReadiUse[™] probenecid buffer:</u> Add **1 ml** of ReadiUse[™] probenecid 25 mM stabilized aqueous solution to **9 ml** of HHBS (1X Hank's with 20 mM Hepes buffer, pH 7.0), and mix them well.

Note1: 10 mL of 2.5 mM ReadiuseTM probenecid buffer is enough for 1 plate. Aliquot and store the unused ReadiuseTM probenecid 25 mM stabilized aqueous solution at < -20 °C, protected from light. Avoid repeated freeze-thaw cycles.

Note2: 2.5 mM ReadiuseTM probenecid buffer contains 0.02% Pluronic[®] F-127, so it is not necessary to add extra Pluronic[®] F-127 in the dye-loading solution.

3 <u>Make dye-loading solution for one cell plate:</u> Add DMSO reconstituted fluorescent calcium dyes (such as Indo-1 AM, Fura-2 AM, Fluo-3 AM, Fluo-4 AM, Fluo-8 AM, Rhod-2 AM and Rhod-4 AM) into 2.5 mM ReadiUseTM probenecid buffer, and mix them well. The working solution is stable for at least 2 hours at room temperature.

Disclaimer: This product is for research use only and is not intended for therapeutic or diagnostic application. Please contact our technical service representative for more information.