Probenecid *Cell Culture Tested*

Ordering Information	*Storage Conditions*
Product Number: 20060 (10x72 mg)	Store at Room Temperature Expiration date is 12 months from the date of receipt

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 good retention of dye indicators inside cells. The use of probenecid to inhibit the activities of transporters, and thus to reduce leakage of intracellular dye indicators is a common method for reducing fluorescence background of calcium assays. Each vial of Cat. # 20060 is dissovled in 0.3 mL of 1 M NaOH, and then diluted into 100 mL of buffer to make the final concentration of probenecid 2.5 mM for your assays. 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. We also offer the convenient ReadiUse™ water-soluble and heat-stable probenecid in the format of powder (Cat. # 20061), solution (Cat. # 20062) or tablet (Cat. # 20063). They are convenient to use and are as effective as the free acid form at the same concentration.

Chemical and Physical Properties

Molecular Weight: 285.36
Solvent: 1 M NaOH

Sample Protocol (for 1 plate)

Note: Following is our recommended protocol. It only provides a guideline, and should be modified according to your specific needs.

1. Dissolve 1 vial of Cat. # 20060 in 0.3 mL of 1 M NaOH, and then diluted into 9.7 mL of HHBS (1X Hank's with 20 mM Hepes buffer, pH 7.0) to make a 25 mM stock solution.
   Note: 1 mL of 25 mM stock solution is enough for 1 plate. Aliquot and store unused stock solution at -20 ^{o}C, protected from light. Avoid repeated freeze-thaw cycles.

2. Make 2.5 mM probenecid buffer: Add **1 mL** of 25 mM probenecid stock solution (from Step 1) to **9 mL** of HHBS with 0.02 to 0.04% Pluronic® F-127, and mix them well.

3. Make dye-loading solution for one cell plate: 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 probenecid buffer (from Step 2), 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.*