Screen Quest™ 10X Calcium Assay Buffer with Phenol Red Plus™

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 36301 (10 plates)	Keep at -20 °C and protect from light	Florescence microplate readers

Introduction

Calcium flux assays are preferred methods in drug discovery for screening G protein coupled receptors (GPCR). Our Screen Quest™ 10X Calcium Assay Buffer with Phenol Red Plus™ contains our water soluble and heat stable probenecid which inhibits the activities of drug-efflux pumps. 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.

Kit Component

Component	Amount
Screen Quest™ 10X Calcium Assay Buffer with Phenol Red Plus™	1 bottle (10 mL)

Protocol (for one plate)

1. Thaw the bottle at room temperature before use.

2. Make 1X Screen Quest ™ calcium assay buffer: Add 1 mL of 10X Screen Quest ™ calcium assay buffer with Phenol Red Plus™ (Cat. # 36301) to 9 mL of HHBS (1X Hank's with 20 mM Hepes buffer, pH 7.0), and mix them well.
   Note: 10 mL of 1X assay buffer is enough for one plate. Aliquot and store unused 10X assay buffer at ≤ -20 °C. Protect from light and avoid repeated freeze-thaw cycles.

3. Make 2X 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 and Fluo-8 AM, Rhod-2 AM and Rhod-4 AM) into 10 mL of 1X Screen Quest ™ calcium assay buffer (from Step 2), to make the final well dye concentration 2X of the desired concentration, and mix them well. The working solution is stable for at least 2 hours at room temperature.

4. To the microplate well add 2X dye-loading solution (from Step 3) which is the same volume as the cell culture medium (*e.g., 100 uL/well/96-well or 25 µL/well/384-well).*

5. Incubate the cells in a 37 °C, 5% CO_2 incubator for about 1 hours, or as desired.

6. Prepare the compound plate with HHBS or your desired buffer.

7. Run the calcium flux assay.

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