MULTISPAN
Assay Experts in GPCRs and Beyond

MULTISCREEN™ Calcium 1.0 No-Wash Assay Kit Protocol

INTRODUCTION

Multiscreen™ Calcium 1.0 No Wash Assay Kits provide homogeneous fluorescence-based assays for intracellular calcium mobilization detection in 96-well, 384-well and higher multiplexity. Pre-loaded with Calcium 1.0 is taken up by the cells where the lipophilic blocking groups of Calcium 1.0 are cleaved by esterases, resulting in a negatively charged fluorescent dye that stays inside cells. The fluorescence signal is greatly enhanced after binding to intracellular calcium released by cell-stimulation. Long wavelength, high sensitivity, and >100 times fluorescence enhancement make Calcium 1.0 an ideal indicator for intracellular calcium release assays measuring GPCRs, calcium channels and receptor tyrosine kinase signaling. Multiscreen™ Calcium 1.0 No Wash Assay Kits are validated by Multiscreen™ stable cell lines.

Kit Components (*Warning: Do not add additional probenecid*)	**Catalog Numbers**			**Storage**	**Instruments**
	MSCA01-1 (1K tests, 384-well)	**MSCA01-10 (10K tests, 384-well)**	**MSCA01-100 (100K tests, 384-well)**		
Component A: Calcium 1.0 + probenecid	1 vial, lyophilized	1 vial, lyophilized	10 vials, lyophilized	-20˚C and avoid light	FLIPR, FDSS NOVOStar FlexStation ViewLux ArrayScan
Component B: 10X Pluronic® F127 Plus	1 bottle (1 mL/bottle)	10 bottles (1 mL/bottle)	10 bottles (10 mL/bottle)		
Component C: HHBS (HANKS + 20 mM HEPES)	1 bottle (9 mL)	1 bottle (100 mL)	Not included		

CALCIUM ASSAY PROTOCOL

1. Prepare cells *(Evaluate each cell line to determine optimal cell density and other conditions.)*

1.1 Adherent: Plate cells overnight in growth media with 10% FBS at 3,000 to 9,000 cells/well/40 µL for a 384-well black poly-D lysine coated clear bottom plate.

1.2 Suspension: Centrifuge the cells from the culture medium and then suspend the cell pellet in the appropriate amount of Component C 10,000 to 20,000 cells/well/30 µL for a 384-well black poly-D lysine coated clear bottom plate. Allow cells to settle for 10 minutes at RT. Centrifuge the plate at 200x g for 5 minutes with brake off prior to the experiments.

2. Prepare Calcium 1.0 dye-loading solution (*Mix well by gentle-vortexing after each step 2.2 - 2.4.)*

2.1 Thaw Component A, Component B and Component C at room temperature.

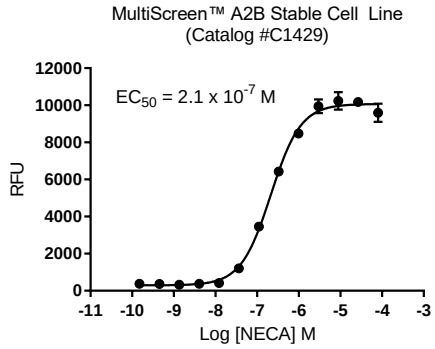
2.2 Make Calcium 1.0 Stock Solution: Add 20 µL of DMSO into the vial of Component A in the MSCA01-1 kit or 200 µL to each vial of Component A in the MSCA01-10 and MSCA01-100 kits. *Mix well.*

2.3 Make 1X Assay Buffer:

- For Catalog MSCA01-1 and MSCA01-10, add 9 mL of Component C into 1 mL Component B. *Mix well*
- For Catalog MSCA01-100, add entire bottle 10mL of Component B into 90 mL of Component C. Prepare in a separate polypropylene bottle (not included in the kit). *Mix well*.

2.4 Make Calcium 1.0 dye-loading solution:

- For Catalog MSCA01-1 and MSCA01-10, add 20 µL of Calcium 1.0 Stock Solution (2.2) to 10 mL of 1X Assay Buffer (2.3). *Mix well.*
- For Catalog MSCA01-100, add 200 µL of Calcium 1.0 Stock Solution (2.2) to 100 mL of 1X Assay Buffer (2.3). *Mix well.*
  - ➢ Aliquot into 10 mL light blocking opaque bottles. Store at -20°C, protect from light, and avoid repeated freeze thaw cycles.
- The final solution is stable for 2 hours at room temperature.



Calcium Dose-Response in MultiScreen™ A2B-HEK293T stable cell line (C1429) measured with MultiScreen™ Calcium 1.0 No Wash Assay Kit. Cells were seeded in assay buffer in a 384-well black wall clear bottom PDL-coated plate, incubated with the MultiScreen™ Calcium 1.0 No Wash Assay Kit before being treated with control agonist NECA and measured by FLIPR.

3. Run Calcium assay:

3.1 For adherent cell assay, replace cell growth media with 30 µL/384-well HHBS buffer to minimize background fluorescence and compound interference by serum.

3.2 Add Calcium 1.0 dye-loading solution at 10 µL/384-well.

3.3 Incubate the dye-loading plate in a cell incubator for 1 hour at 37˚C.

3.4 Prepare compound plate with Component C

3.5 Run Calcium flux assay by monitoring the fluorescence intensity at Ex/Em = 490/ 525 nm.

Note: Prepare instrument by adjusting the signal intensity to 10%-15% of the maximum instrument counts. For example, the maximum for FLIPR-384 is 65,000, so the settings should be adjusted to 7,000 to 10,000.

26219 Eden Landing Road, Hayward, California 94545-3718 Phone: (510) 887-0817 info@multispaninc.com