MULTISPAN
Assay Experts in GPCRs and Beyond

MULTISCREEN™ Calcium 1.0 No-Wash Assay Kit Protocol

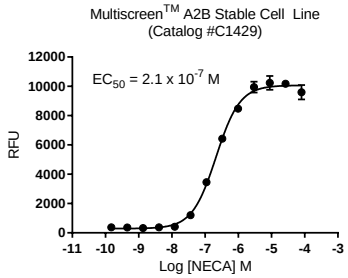
INTRODUCTION

Multiscreen™ Calcium 1.0 No Wash Assay Kits provide homogeneous fluorescence-based assays for intracellular calcium mobilization detection. Pre-loaded with Calcium 1.0 is taken up by the cell 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. The assay can be performed in 96- or 384-well microtiter-plate format and adapted to automation.

Kit Components (*Warning: Do not add additional probenecid*)	**Catalog Numbers**			**Storage**	**Instrument Platform**
	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-vortexting 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: Add 9 mL of Component C to 1 mL Component B for MSCA01-1 kit, 90 mL of Component C to 10 mL Component B for MSCA01-10 or 900mL HHBS buffer (not included) to 100 mL Component B for MSCA01-100. *Mix well*
   - 2.4 Make Calcium 1.0 dye-loading solution: Add 20 µL of Calcium 1.0 Stock Solution (2.2) to 10 mL of 1X Assay Buffer (2.3) or scale up proportionally. *Mix well.* The final solution is stable for 2 hours at room temperature.
3. **Run Calcium assay:**
   - 3.1 Replace cell growth media with 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.



Sample Data: Calcium Dose Response Curve in Multiscreen™ A2B-HEK293T stable cell line (Multispan C1429)

A2B-HEK293T cells were resuspended in HHBS buffer at 10K cells per 30µL per well in a 384-well black clear bottom PDL-coated plate. The cells were subsequently incubated for 1 hour at 37°C with 10µL of the Calcium 1.0 Loading Solution made from Multiscreen™ Calcium 1.0 No Wash Assay Kit before being treated with Control Agonist NECA at 10µL/well, added and measured by FLIPR.

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