MULTISPAN
Assay Experts in GPCRs and Beyond

MULTISCREEN™ TR-FRET cAMP 1.0 No-Wash Assay Kit

INTRODUCTION

Multiscreen™ TR-FRET cAMP 1.0 No Wash Assay Kits provide a homogenous TR-FRET assay method for adenylyl cyclase activity detection in 96-well, 384-well and higher multiplexity. α-cAMP antibody (Ab) is labeled with MultiScreen™Eu while cAMP is labeled with MultiScreen™650. In the absence of cAMP, MultiScreen™650-cAMP is bound to MultiScreen™Eu-α-cAMP-Ab to give a strong TR-FRET Emission at 655 nm. Free cAMP in the test sample competes for binding to the MultiScreen™ Eu-α-cAMP-Ab, reducing TR-FRET signal from MultiScreen™650-cAMP binding. The MultiScreen™650-cAMP only has fluorescence lifetime of nanosecond while MultiScreen™Eu-α-cAMP-Ab has much longer fluorescence lifetime value due to TR-FRET. The magnitude of TR-FRET is proportional to the concentration of cAMP in a sample. Multiscreen™ TR-FRET cAMP 1.0 No Wash Assay Kits are validated by Multiscreen™ stable cell lines.

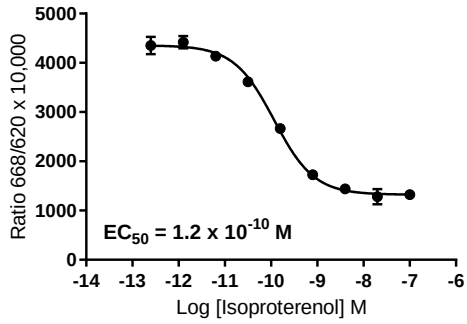
Kit Components	Catalog Numbers			Storage	Instrument Platform
	MSCM01-1 (0.5K tests, 384-well)	MSCM01-10 (5K tests, 384-well)	MSCM01-100 (25K tests, 384-well)		
Component A: MultiScreen™Eu-α-cAMP-Ab	1vial	1 vial 50x	5 vials 50x	-4˚C and avoid light	TR-FRET microplate readers
Component B: MultiScreen™650-cAMP	1vial	1 vial 50x	5 vials 50x		
Component C: cAMP Standard	1 vial (20 µL 1mM)	1 vial (33 µg)	1 vial (33 µg)		
Component D: Cell Lysis Buffer	10 mL	100 mL	100 mL 5 bottles		
Component E: Diluent	10 mL	100 mL	100 mL 5 bottles		
Reagents NOT included in the kit	Assay Buffer: Hank's Balanced Salt Solution with 20mM HEPES, pH 7.4; When necessary: IBMX (3-Isobutyl-1-methylxanthine); Forskolin (Adenylyl cyclase activator)				

PREPARE WORKING SOLUTIONS (*Mix well by gentle-vortexing or pipette mixing after each step)*:

1. **cAMP standard:** Add 100 µL Component E to Component C to make 1mM stock solution for MSCM01-10 and MSCM01-100 kits only and gently vortex to mix: Add 1 µL 1mM stock solution into 99 µL Component E or cell culture media to make 10µM standard followed 4-fold serial dilution to make 10000, 2500, 625, 156.3, 39.1, 9.8, 2.4, 0.61, 0.15, 0.038 nM final concentrations. Mix gently with a pipette after each dilution. Add 10µL or 20µL of serial diluted cAMP standard per 384-well in microtiter assay plate after last the incubation period from step 5.
2. **MultiScreen™Eu-α-cAMP-Ab working solution:** Add 50µL Component A to 2.5mL Component D (scale down based on need). Prepare right before use. Store at 4ºC.
3. **MultiScreen™650-cAMP working solution:** Add 50µL Component B to 2.5mL Component D (scale down based on need). Prepare right before use. Store at 4ºC.

cAMP ASSAY PROTOCOL (384-well format)

4. **Prepare cells** (*Evaluate each cell line to determine optimal cell density and other conditions.)*
   Adherent: Plate cells overnight in growth media with 10% FBS at 3k-9k cells/40µL/384-well in Poly-D-Lysine coated white opaque bottom plate. Remove growth media carefully before compound treatment.
   Suspension: Centrifuge the cells from the culture media and then suspend the cell pellet in the appropriate amount of Assay Buffer at 3k-12k cells/5 µL/ 384-well in small volume, white, opaque bottom plate.
5. **Compound Treatment (***The incubation time and temperature can be optimized for each receptor***):** Add 15µL of test compounds to adherent cells or 5µL to suspension cells per well and incubate for 20 minutes at 37ºC.
6. **Termination (30µL or 20µL final volume):** Add 7.5µL MultiScreen™650-cAMP working solution and 7.5µL MultiScreen™Eu-α-cAMP-Ab working solution sequentially to adherent cells per well or 5µL of each sequentially to suspension cells per well. Incubate 30 minutes at room temperature in the dark. Read fluorescence emission on a TR-FRET compatible reader at 665 nm and 620 nm.



cAMP Dose-Response in MultiScreen™ β1-HEK293T stable cell line (C1437-1) measured with MultiScreen™ TR-FRET cAMP 1.0 No Wash Assay Kit. Cells were seeded in a 384-well solid white plate, incubated with Isoproterenol and 1mM IBMX before being treated with MultiScreen™Eu-α-cAMP-Ab and MultiScreen™650-cAMP, and measured by FlexStation 3.

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