Screen Quest™ Coelenterazine Calcium Assay Kit

Ordering Information:
Product Number: #36305 (10 plates), #36306 (100 plates).

Instrument Platform:
Luminescence micro-plate readers

Storage Conditions:
Keep in freezer and avoid light.

Introduction

Calcium flux assays are preferred methods in drug discovery for screening G protein coupled receptors (GPCR). This kit uses a highly calcium-sensitive and membrane-permeable coelenterazine analog as a calcium indicator for the cells that are transfected with apoaequorin gene. Aequorin is a calcium-sensitive bioluminescent protein from the jellyfish Aequorea victoria that has been used extensively as a calcium indicator in cells. The aequorin complex emits blue light when bound to calcium ions. The luminescence intensity is directly proportional to the Ca2+ concentration. Our coelenterazine–based kit is much more sensitive than the fluorescence-based calcium assay kits (such as Fluo-4, Fluo-3, Calcium-3 and Calcium-4). This kit provides an optimized assay method for monitoring G-protein-coupled receptors (GPCRs) and calcium channels. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation. It might be useful for monitoring of intracellular calcium mobilization in a specified compartment given that recombinant apoaequorin proteins can now be targeted to specific organelles, cells and tissues.

Kit Key Features

Increased Signal to Background Ratio: The Coelentrazine analog in the kit is more sensitive than the fluorescence-based calcium assay kits indicator, with much higher Signal/Background ratio due to very low luminescence background.

Convenient and Robust: Formulated to have minimal hands-on time. No wash step needed.

Versatile applications: Compatible with many cell lines and targets without ligand or target interference.

Kit Components

Materials	#36305 (10 plates)	#36306 (100 plates)
Component A: Coelenterazine analog	1 vial, lyophilized	10 vials, lyophilized
Component B: 100% ETOH	1 vial (500 µL)	1 bottles (5 mL)
Component C: Assay buffer	1 bottle (100 ml—1X ready to use)	1 bottle (100 ml—10X)

Materials Required (but not provided)

- 96 or 384-well microplate: Tissue culture microplate with white wall and clear bottom.
- Luminecence microplate readers
- HHBS (1X Hank's with 20 mM Hepes buffer, pH 7.0).

Assay Protocol (for 1 plate)

Brief Summary

Prepare cells → Take medium off → Add coelenterazine-loading solution (100 µL for 96-well-plate or 25 µL for 384-well-plate) → Incubate at room temperature for 3-4 hrs → Monitor the aequorin luminescence

1. Prepare Cells

1.1 For adherent cells, plate cells overnight in growth medium at 40,000 to 80,000 cells/well/100µL for 96-well or 10,000 to 20,000 cells/well/25µL for 384-well plates.

1.2 For non-adherent cells, centrifuge the cells from the culture medium and then suspend the cell pellets in coelenterazine -loading solution (see steps 2.4) at 125,000 to 250,000 cells/well/100µL for 96-well or 30,000 to 60,000 cells/well/25µL for 384-well poly-D lysine plates. Centrifuge the plates at 800 rpm for 2 minutes with break off prior to the experiments
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for the intracellular calcium mobilization.

2. Prepare coelenterazine -loading solution (for 1 plate)

2.1 Thaw all the components at room temperature before use.

2.2 Make coelenterazine analog by adding 250 µL component B (100% ETOH) into component A (Coelenterazine analog), mixing them well.
Note: 25 µL of reconstituted coelenterazine analog is enough for 1 plate, un-used reconstituted *coelenterazine analog stock solution can be stored at ≤ -20°C for more than one month if the tubes are sealed tightly, avoiding light and repeated freeze-thaw cycles.*

2.3 Make 1X assay buffer
a). For **Cat# 36305 (10 plates kit)**, ready to use 1X assay buffer (Component C).
b). For **Cat# 36306 (100 plates kit)**, make 1X assay buffer by diluting 10 mL of component C (10 X assay buffer) into **90 mL** HHBS buffer (not included in the kit), mix them well.
Note: 10 mL 1X assay buffer is enough for 1 plate, store un-used 1X assay buffer at 4°C.

2.4 Make coelenterazine-loading solution for one cell plate by adding 25 µl of ETOH reconstituted coelenterazine analog (from step 2.2) into 10 mL of 1X assay buffer (from step 2.3), mixing them well, protect from light. This working solution is stable for at least 2 hours at room temperature.

3. Run Calcium Assay

3.1 Remove the growth medium from the cell plates

Note1: It is important to remove the growth medium in order to minimize compound interference with serum or culture media.
Note2: Alternatively, one can grow the cells in growth medium with 0.5-to 1% FBS to avoid medium removal step, in this case, make 2X coelenterazine- loading solution in 1X assay buffer.

3.2 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) coelentrazine loading solution into the cells.
3.3 Incubate the coelentrazine-loading plates at room temperature for 3-4 hours, protect from light.
3.4 Prepare the compound plates by using HHBS or your desired buffer.
3.5 Monitor the aequorin luminescence by using photon detection system that has an enclosed chamber containing a photomultiplier; the instrument must completely exclude outside light.

Data Analysis

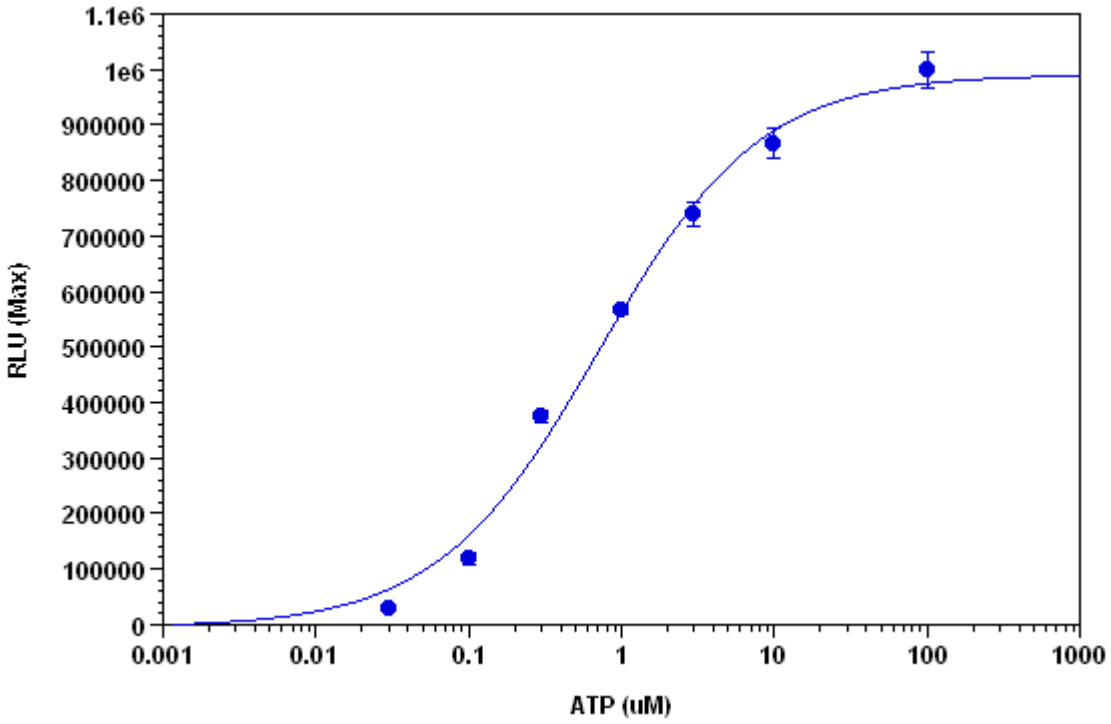

Figure 1. **ATP Dose Response on CHO-aeq.** CHO cells stably transfected with apoaequrin were seed overnight in 50,000 cells per 100 µL per well in a 96-well white wall/clear bottom Costar plate. The growth medium was removed and the cells were incubated with 100 µL of the Screen Quest™ Coelenterazine Calcium Assay Kit for 3 hr at room temperature with light protection. ATP (25 µL/well) was added by by NOVOstar (BMG LabTech) to achieve the final indicated concentrations. The EC_{50} of ATP is about 0.8 µM.

Related Products

21013	Fluo-3, AM *Custom packaging*	20x50 µg
21011	Fluo-3, AM *UltraPure grade*	1 mg
21021	Fura-2, AM *UltraPure Grade*	1 mg
21036	Indo-1, AM *Custom packaging*	20x50 µg
21032	Indo-1, AM *UltraPure Grade*	1 mg
20053	Pluronic® F-127 *10% solution in water*	10 mL
20052	Pluronic® F-127 *20% solution in DMSO*	10 mL
20060	Probenecid *Cell culture tested*	10x150 mg
20061	Probenecid *Water-soluble*	10x150 mg
21062	Rhod-2, AM *UltraPure Grade*	1 mg
21063	Rhod-2, AM *UltraPure Grade* *Bulk packaging*	50 mg
21064	Rhod-2, AM *UltraPure Grade* *Custom packaging*	20x50 µg
21070	Rhod-5N, AM	1 mg
21080	Quest Fluo-8™ AM *Cell-permeable*	1 mg
21081	Quest Fluo-8™, AM *Cell-permeable*	5x50 µg
21082	Quest Fluo-8™, AM *Cell-permeable*	10x50 µg
21083	Quest Fluo-8™, AM *Cell-permeable*	20x50 µg
21090	Quest Fluo-8H™, AM *Cell-permeable*	1 mg
21091	Quest Fluo-8H™, AM *Cell-permeable*	10x50 µg
21096	Quest Fluo-8L™, AM *Cell-permeable*	1 mg
21097	Quest Fluo-8L™, AM *Cell-permeable*	10x50 µg
21120	Quest Rhod-4™, AM	1 mg
21121	Quest Rhod-4™, AM	5x50 ug
21122	Quest Rhod-4™, AM	10x50 ug
21123	Quest Rhod-4™, AM	20x50 µg
36301	Screen Quest™ 10X calcium assay buffer	10 Plates
36302	Screen Quest™ 10X calcium assay buffer	100 Plates
36303	Screen Quest™ 10X calcium assay buffer with Phenol Red Plus™	10 Plates
36305	Screen Quest™ Coelenterazine Calcium Assay Kit *10 Plates*	1 kit
36306	Screen Quest™ Coelenterazine Calcium Assay Kit *10X10 Plates*	1 kit
36307	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal*	1 Plate
36308	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal*	10 Plates
36309	Screen Quest™ Fluo-8 NW Calcium Assay Kit *Medium Removal*	100 Plates
36314	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium*	1 Plate
36315	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium *	10 Plates
36316	Screen Quest™ Fluo-8 NW Calcium Assay Kit *1% FBS Growth Medium*	100 Plates
36330	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal*	1 Plate
36331	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal*	10 Plates
36332	Screen Quest™ Rhod-4 NW Calcium Assay Kit *Medium Removal*	100 Plates
36333	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium*	1 Plate
36334	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium *	10 Plates
36335	Screen Quest™ Rhod-4 NW Calcium Assay Kit *1% FBS Growth Medium*	100 Plates
2450	Trypan Blue, sodium salt *Cell culture tested*	100 g
2456	Trypan Red Plus™, sodium salt *0.1 M aqueous solution*	10 mL
2457	Trypan Red Plus™, sodium salt *0.1 M aqueous solution*	100 mL
2455	Trypan UltraBlue™, sodium salt	1 g

www.interchim.com
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211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com