Screen Quest™ Luminometric Calcium Assay Kit

Catalog number: 36305, 36306
Unit size: 10 Plates, 100 Plates

Component	Storage	Amount	
		Cat No. 36305	Cat No. 36306
Component A: Coelenterazine Analog	Freeze (<-15 °C), Minimize light exposure	1 vial, lyophilized	10 vials, lyophilized
Component B: 100% ETOH	Freeze (<-15 °C), Minimize light exposure	1 vial (500 µL)	1 bottle (5 mL)
Component C: Assay Buffer	Refrigerate (2-8 °C)	1 bottle (100 mL-1X ready to use)	1 bottle (100 mL-10X)

OVERVIEW

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.

AT A GLANCE

Protocol summary

1. Prepare cells by removing growth medium
2. Add Coelenterazine-loading solution (100 µL/well for 96-well plate or 25 µL/well for 384-well plate)
3. Incubate at room temperature for 3-4 hours
4. Monitor aequorin luminescence intensity

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Luminescence microplate reader
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode/Programmable liquid handling

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Coelenterazine analog:
Add 250 µL of 100% ETOH (Component B) into the vial of Coelenterazine analog (Component A), and mix them well.

Note 25 µL of reconstituted coelenterazine analog is enough for one plate. Unused coelenterazine analog stock solution can be stored at < -20 °C for more than one month if the tubes are sealed tightly. Protect from light and avoid repeated freeze-thaw cycles.

2. Assay Buffer (1X):
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 10X Assay Buffer (Component C) into 90 mL of HHBS buffer (not included in the kit), and mix them well.

Note 10 mL of 1X assay buffer is enough for one plate. Store unused 1X assay buffer at 4 °C.

PREPARATION OF WORKING SOLUTION

Coelenterazine-loading solution:
Add 25 µL of ETOH reconstituted coelenterazine analog (Prepartion Of Stock Solutions) into 10 mL of 1X assay buffer (Preparation Of Stock Solutions), and mix them well.

Note This working solution is stable for at least 2 hours at room temperature, protected from light.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Remove the growth medium from the cell plates.

   Note It is important to remove the growth medium in order to minimize compound interference with serum or culture media.

   Note Alternatively, grow the cells in growth medium with 0.5-1% FBS to avoid medium removal step. In this case, 2X Coelenterazine-loading solution in 1X buffer is needed.

2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) Coelenterazine-loading solution into the cell plates.

3. Incubate the Coelenterazine-loading plates at room temperature for 3-4 hours, protected from light.

4. Prepare the compound plates with HHBS or the desired buffer.

5. Monitor the aequorin luminescence intensity by using the photon detection system that has an enclosed chamber containing a photomultiplier. The instrument must completely exclude outside light.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RLU (Max)) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate ATP samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

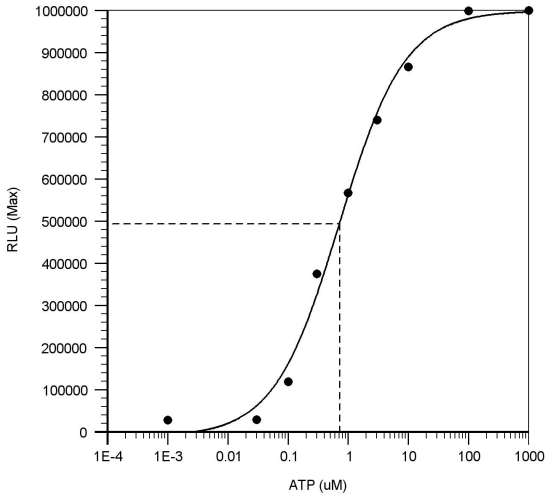

Figure 1. ATP Dose Response on CHO-aeq cells. CHO cells stably transfected with apoaequrin were seeded overnight at 50,000 cells/100 µL/well in a Costar white wall/clear bottom 96-well plate. The growth medium was removed and the cells were incubated with 100 µL of dye-loading solution using the Screen Quest™ Coelenterazine Calcium Assay Kit for 3 hours at room temperature and protected from light. ATP (25 µL/well) was added by NOVOstar (BMG Labtech) to achieve the final indicated concentrations. The EC50 of ATP is about 0.8 µM.