# Screen Quest™ Luminometric Calcium Assay Kit

| *Ordering Information* | *Storage Conditions* | *Instrument Platform* |
|---|---|---|
| Product Number: 36305 (10 plates), 36306 (100 plates) | Keep in freezer and protect from light | Luminescence microplate readers |

## Introduction

Calcium flux assays are preferred methods in drug discovery for screening G protein coupled receptors (GPCR). These kits use 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 $Ca^{2+}$ concentration. Our coelenterazine-based kits are much more sensitive than the fluorescence-based calcium assay kits (such as Fluo-4, Fluo-3, Calcium-3 and Calcium-4). These kits provide an optimized assay method for monitoring the G-protein-coupled receptors 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 the intracellular calcium mobilization in a specified compartment given that recombinant apoaequorin proteins can now be targeted to specific organelles, cells and tissues. This kit is more sensitive than the fluorescent calcium assays.

### Kit Key Features

| | |
|---|---|
| ***Increased S/B Ratio:*** | High signal to background ratio with very low luminescence background. |
| ***Convenient:*** | 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

| **Components** | **Amount** | |
|---|---|---|
| | **Cat. # 36305 (10 plates)** | **Cat. # 36306 (100 plates)** |
| Component A: Coelenterazine Analog | 1 vial, lyophilized | 10 vials, lyophilized |
| Component B: 100% ETOH | 1 vial (500 µL) | 1 bottle (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 microplates: Tissue culture microplates with white wall and clear bottom
- A luminecence microplate reader
- HHBS (1X Hank's with 20 mM Hepes Buffer, pH 7.0)

## Assay Protocol for One 96-Well Plate

### Brief Summary

**Prepare cells → Remove growth medium → Add coelenterazine-loading solution (100 µL /well/96-well plate or 25 µL/well/384-well plate) → Incubate at room temperature for 3-4 hours → Monitor aequorin luminescence intensity**

**1. Prepare cells:**

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

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

**2. Prepare coelenterazine -loading solution:**

2.1 Thaw all the kit components at room temperature before use.

2.2 Make 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 $^{o}$C for more than one month if the tubes are sealed tightly. Protect from light and avoid 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 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 $^{o}$C.*

2.4 Make coelenterazine-loading solution for one cell plate: Add 25 µL of ETOH reconstituted coelenterazine analog (from Step 2.2) into 10 mL of 1X assay buffer (from Step 2.3), and mix them well. This working solution is stable for at least 2 hours at room temperature, protected from light.

**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, 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 assay buffer is needed.*

3.2 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) coelenterazine loading solution (from Step 2.4) into the cell plates.

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

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

3.5 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.

## Data Analysis

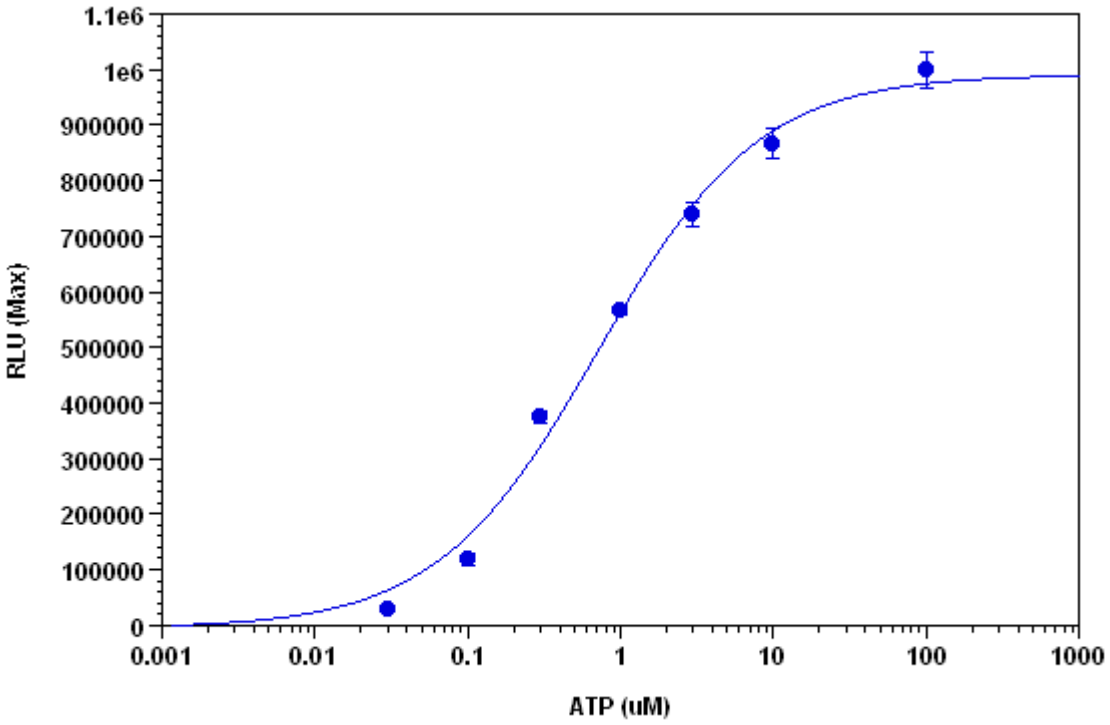


**Figure 1.** ATP Dose Response on CHO-aeq cells. CHO cells stably transfected with apoaequrin were seeded overnight at 50,000 cells/100 µL/well in a Costar white wall/clear bottom 96-well plate. The growth medium was removed and the cells were incubated with 100 µL of dye-loading solution using the 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 $EC_{50}$ of ATP is about 0.8 µM.