ab112114

Calcium Detection Kit (Luminometric)

Instructions for Use

For monitoring G-protein-coupled receptors (GPCRs) and calcium channels using a coelenterazine analog as a calcium indicator for the cells that are transfected with apoaequorin gene

View kit datasheet: www.abcam.com/ab112114
(use www.abcam.cn/ab112114 for China, or www.abcam.co.jp/ab112114 for Japan)

This product is for research use only and is not intended for diagnostic use.

Version 3 Last Updated 19 December 2018

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1. 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 Ca^{2+} concentration.

ab112114 Calcium Detection Kit – Luminometric is much more sensitive than the fluorescence-based calcium assays. This kit provides 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. ab112114 is more sensitive than the fluorescent calcium assays.

Kit Key Features

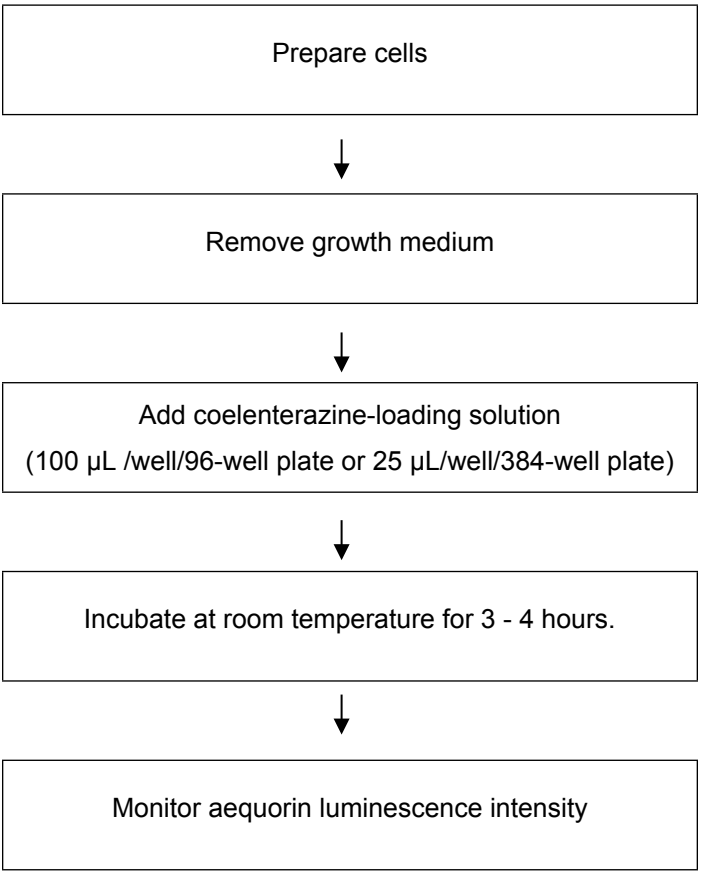
- **Increased S/B Ratio:** High signal to background ratio with very low luminescence background.

- **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.

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Item	10 x 96 tests	100 x 96 tests
Coelenterazine Analog	1 vial (lyophilized)	10 vials (lyophilized)
100% ETOH	1 x 500 µL	1 x 5000 µL
Assay Buffer	1 x 100 mL (1X ready to use)	1 x 100 mL (10X)

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Additional Materials Required

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

6. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Prepare Cells

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.

2. For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellet in the coelenterazine - loading solution (see section 2, step 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

B. Prepare coelenterazine - loading solution

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

2. Make coelenterazine analog: Add 250 µL of 100% ETOH into the vial of Coelenterazine Analog, 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.

3. Make 1X assay buffer: Ready to use 1X Assay Buffer supplied.
   Note: 10 mL of 1X assay buffer is enough for one plate. Store unused 1X assay buffer at 4 °C.

4. Make coelenterazine - loading solution for one cell plate: Add 25 µL of ETOH reconstituted coelenterazine analog (from Step 2) into 10 mL of 1X assay buffer (from Step 3), and mix them well. This working solution is stable for at least 2 hours at room temperature, protected from light.

C. Run Calcium Assay:

1. Remove the growth medium from the cell plates.

Note 1: It is important to remove the growth medium in order to minimize compound interference with serum or culture media.

Note 2: 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.

2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) coelenterazine loading solution (from section B, step 4) 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.

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.

7. Data Analysis

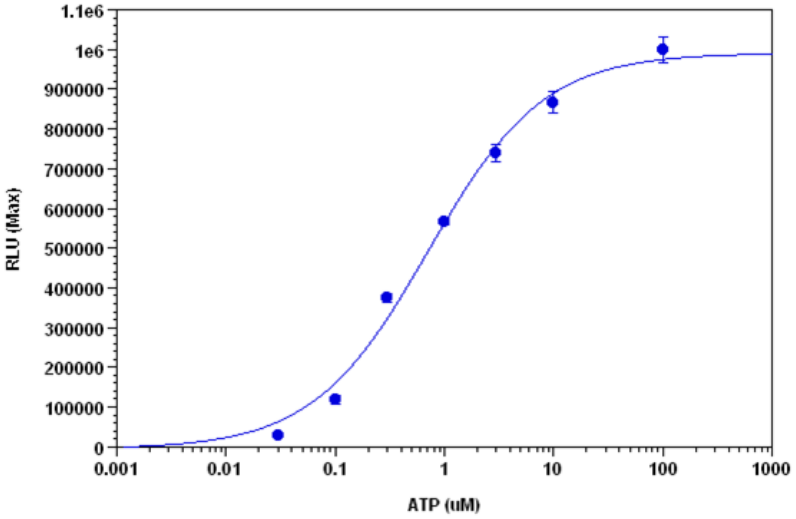


Figure 1. ATP Dose Response on CHO-aeq cells. CHO cells stably transfected with apoaequrin were seeded overnight at 50,000 cells/100 µL/well in a white wall/clear bottom 96-well plate. The growth medium was removed and the cells were incubated with 100 µL of dye-loading solution using the ab112114 for 3 hours at room temperature and protected from light. ATP (25 µL/well) was added to achieve the final indicated concentrations. The EC_{50} of ATP is about 0.8 µM.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

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