ab176767

Chloride Channel Assay Kit (Colorimetric)

Instructions for Use

An optimized assay for monitoring chloride channels.

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

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1. Overview

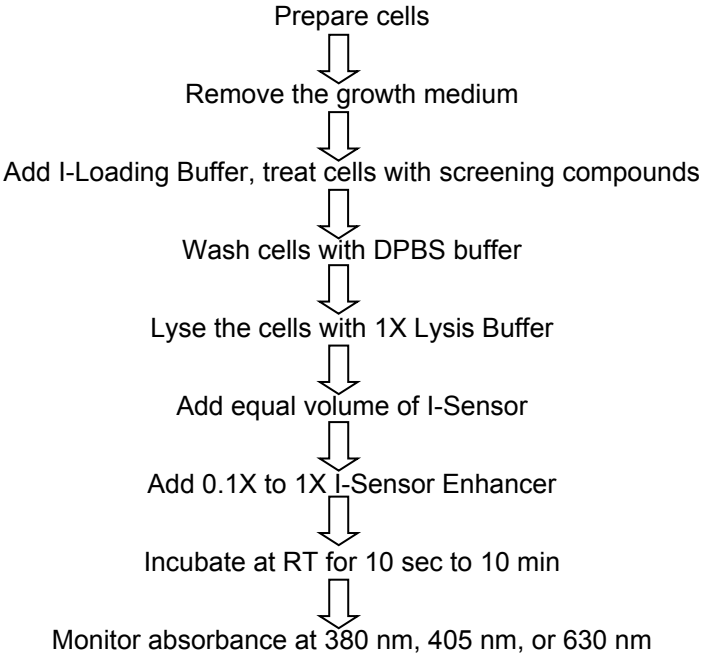
Chloride channels have a variety of important physiological and cellular functions that include regulation of pH, volume homeostasis, organic solute transport, cell migration, cell proliferation and differentiation. Chloride channels represent valuable drug targets. A number of chronic disease states such as cystic fibrosis and Bartter's syndrome are due to defects in chloride channel functions. However, the existing technologies for screening chloride channel modulators are a compromise between throughput, sensitivity and physiological relevance.

Abcam’s Chloride Channel Assay Kit (Colorimetric) (ab176767) provides a sensitive and robust colorimetric method for studying chloride channels. The assay is based on our proprietary iodide indicator (Iodide Sensor Blue dye) to measure iodide concentration, as low as 30 nM of iodide can be detected. Iodide Sensor Blue dye forms a blue complex with iodide, which has absorption spanning from the UV to 700 nm. Thus a few absorption wavelengths can be used for monitoring the iodide-dependent color change. Abcam's Chloride Channel Assay Kit (Colorimetric) (ab176767) provides an optimized assay method for monitoring chloride channels. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format.

Kit Key Features

- **High sensitivity** – As low as 30 nM of iodide can be detected
- **Convenient** – Formulated to have minimal hands-on-time
- **Less toxicity** – Compared to the classic Sandell and Kolthoff assay
- **Continuous** – Continuous assay without a separation step

2. Protocol Summary



3. Kit Components

Item	Quantity	Storage upon arrival	Storage after use/ reconstitution
Iodide Sensor Blue dye	50 mL	4°C	4°C
100X Iodide Sensor Enhancer	0.5 mL	4°C	4°C
I-Loading Buffer	100 mL	4°C	4°C
10X Cell Lysis Buffer	5 mL	4°C	4°C

4. Storage and Stability

Upon arrival, store the kit at 4°C and protected from light. Please read the entire protocol before performing the assay.

Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- 96 or 384-well black plate with clear flat bottoms
- Centrifuge
- Multi-well spectrophotometer (ELISA reader)
- Sterile water
- DPBS
- HBSS

6. Assay Protocol

1. Prepare cells:

a) For adherent cells

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

b) For non-adherent cells

Centrifuge the cells from the culture medium and then suspend the cell pellet in pre-warmed assay buffer at 125,000 to 250,000 cells/well/100µL 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 an individual basis to determine the optimal cell density.

2. Prepare iodide assay reagents:

a) Warm all the reagents to room temperature before use.

b) Make 1X I-Sensor Enhancer solution: Add 50 µL of100X Iodide Sensor Enhancer to 5 mL of sterile H_2O, and mix them well.

Note1: 1X I-Sensor Enhancer solution is not stable, use within 2 hours after the dilution.

Note2: Each cell line should be evaluated on an individual basis to determine the optimal dilution of I-Sensor Enhancer solution. We noticed that 0.1X I-Sensor Enhancer solution works even better for some cell lines.

c) Make 1X cell lysis buffer: Add the whole vial of 10X Cell Lysis Buffer to 45 mL of sterile H_2O, and mix them well.

Note: 5 mL of 1X cell lysis buffer is enough for one plate. Store unused 1X cell lysis buffer at 4°C.

3. For iodide efflux assay:

a) Aspirate the growth medium from the cell plate.

b) Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of pre-warmed I-Loading Buffer and incubate for 2-4 hours at 37 °C.

c) Aspirate the iodide loading buffer completely, and wash the cells with DPBS or HBSS at least 3 times.

d) Treat the cells with agonist in HBSS buffer for 5 minutes at 37 °C.

Note: For antagonists screen, incubate the compounds with I-Loading Buffer for at least an additional 30 min before the cells were washed with DPBS or HBSS buffer.

e) Aspirate the supernatant and wash the samples with DPBS or HBSS at least 3 times.

f) Lyse the cells by adding 50 µL/well (96-well plate), or 25 µL/well (384-well plate) of 1X cell lysis buffer (from Step 2c).

g) Perform the iodide assay (See Step 5).

4. For iodide influx assay:

a) Aspirate the growth medium from the cell plate.

b) Add 100 µL/well (96-well plate), or 25 µL/well (384-well plate) of pre-warmed I-Loading Buffer with test compounds, and incubate for 5 minutes.

c) Aspirate the iodide loading buffer completely, and wash the cells with HBSS 3 times.

d) Lyse the cells by adding 50 µL/well (96-well plate), or 25 µL/well (384-well plate) of 1X cell lysis buffer (from Step 2c).

e) Perform the iodide assay (See Step 5).

5. Run iodide assay:

a) Add 50 µL/well (96-well plate), or 25 µL/well (384-well plate) of Iodide Sensor Blue dye to the wells that contain different concentrations of potassium iodide (from Step 3g or Step 4e).

b) Add 50 µL/well (96-well plate), or 25 µL/well (384-well plate) of 1X Iodide Sensor Enhancer solution (from Step 2b) into the mixture (Step 5a).

Note: For some cell lines, you might need to dilute enhancer solution down to 0.1X.

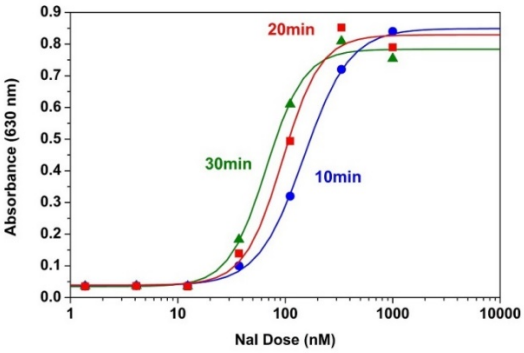
c) Incubate at room temperature for 10 sec-10 min.

Note1: Each cell line should be evaluated on an individual basis to determine the optimal incubation time.

Note2: The blue color may change to yellow within seconds to minutes due to the presence of a high concentration of iodide.

d) Monitor absorbance at 630, 380, or 405 nm.

7. Data Analysis



NaI dose response was measured with Abcam's Chloride Channel Assay Kit (Colorimetric) (ab176767) in a 96-well black wall/clear bottom plate. As low as 30 nM of NaI was detected with 10 minutes incubation time (n = 3).

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)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	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	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)

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