Screen Quest™ Colorimetric Chloride Channel Assay Kit

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: #36350 (10 plates)	Keep at 4°C and avoid light	All absorbance bottom-reading microplate readers with proper filters

Introduction

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 diseases 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. Screen Quest™ Colorimetric Chloride Channel Assay Kit provides a sensitive and robust colorimetric method for studying chloride channels. The assay is based on our proprietary iodide indicator (Iodide Blue™) for measuring iodide concentration, as low as 30 nM of iodide can be detected. Screen Quest™ Chloride Channel Assay Kit provides an optimized assay for monitoring chloride channels. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation.

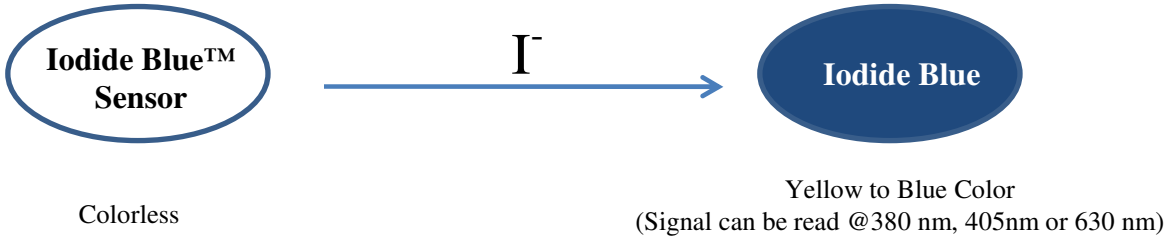

Figure 1: Principle of Screen Quest™ Colorimetric Chloride Channel Assay Kit

Kit Features and Benefits

High Sensitivity:	As low as 30 nM iodide can be detected.
Convenient and Robust:	Formulated to have minimal hands-on time.
Less toxicity:	Ease of use with less toxicity than the classic Sandell and Kolthoff assay.
Continuous:	Continuous assay without separation required.

Kit Components

Components	Amount
Component A: Iodide Blue™ Sensor	1 bottle (50 mL)
Component B: Iodide Sensor Enhancer (100X)	1 vial (0.5 mL)
Component C: I^- Loading Buffer	1 bottle (100 mL)
Component D: Cell Lysis Buffer (10X)	1 bottle (5 mL)

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Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells → Take medium off → Add I^- loading buffer, treat cells with screening compounds → Wash cells with DPBS buffer 3 times → Lysis the cells with 1X lysis buffer (50 µL for 96-well-plate or 25 µL for 384-well-plate) → Add equal volume of I^- sensor (50 or 25 µL) → Add 0.1X to 1X I^- sensor enhancer (50 or 25 µL) → Incubate at room temperature for 10 sec to 10 min → Read absorbance at 380 nm, 405 nm, or 630 nm

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 pre-warmed assay buffer 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.

2. Prepare iodide assay reagents (for 1 plate):

2.1 Warm up all the reagents to room temperature before use.

2.2 Make 1X I^- sensor enhancer solution by adding 50 µL of I^- sensor enhancer (100X) (Component B) to 5 mL of distilled H_2O, mixing them well.
Note1: 1X reconstituted I^- sensor enhancer solution is not stable, use it 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 noted that 0.1 X I^- sensor enhancer solution works even better for some cell lines.

2.3 Make 1X cell lysis buffer by adding whole vial of Cell Lysis Buffer (10X) (Component D) to 45 mL of disteril H_2O, mixing them well.
Note: 5 mL1X cell lysis buffer is enough for 1 plate, store unused 1X cell lysis buffer at 4°C.

3. For iodide efflux assay:

3.1 Aspirate the growth medium from the cell plate.

3.2 Add 100 µL/ well (96-well plate), or 25 µL/ well (384-well plate) of pre-warmed I^- Loading Buffer (Component C) for 2- 4 hours.

3.3 Aspirate the iodide loading buffer completely, and wash the cells with DPBS at least 3 times.

3.4 Treat the cells with agonist in DPBS or HBSS buffer or buffer of your choice for 5 minutes.
Note: For antagonists screen, incubate the compounds with I^- loading buffer for a minimum of an additional 30 min before the cells were washed with DPBS buffer.

3.5 Aspirate the supernatant.

3.6 Lysis the cells by adding 50 µL/ well (96-well plate), or 25 µL/ well (384-well plate) of 1X cell lysis buffer (from step 2.3).

3.7 Perform the iodide assay (See step 5).

4. For iodide influx assay:

4.1 Aspirate the growth medium from the cell plate.

4.2 Add 100 µL/ well (96-well plate), or 25 µL/ well (384-well plate) of pre-warmed I^- Loading Buffer (Component C) with test compounds for 5 minutes.

4.3 Aspirate the iodide loading buffer completely, and wash the cells with DPBS 3 times.

4.4 Lysis the cells by adding 50 µL/ well (96-well plate), or 25 µL/ well (384-well plate) of 1X cell lysis buffer (from step 2.3)

4.5 Perform the iodide assay (See step 5)

5. Run iodide assay:

5.1 Add 50 µL/ well for 96-well plate, or 25 µL/ well for 384-well plate of Iodide Blue™ sensor (Component A) to the wells that contain different concentrations of potassium iodide (from step 3.7 or step 4.5).

5.2 Add 50 µL/ well (96-well plate), or 25 µL/ well (384-well plate) of 1X iodide sensor enhancer solution (from step 2.2) to step 5.1.
Note: For some cell lines you might need to dilute enhancer solution down to 0.1X.

5.3 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.

5.4 Read absorbance at 630, 380, or 405 nm.

Data Analysis

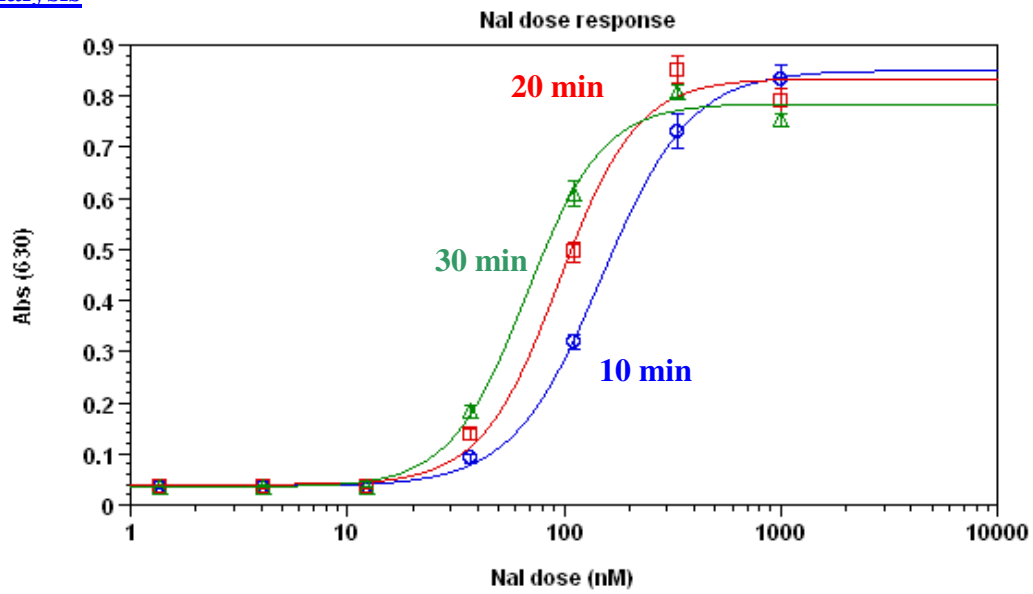

Figure 2. NaI dose response on 96-well black plate was measured with Screen Quest™ Chloride Channel Assay Kit. As low as 30 nM (10 pmol/well) of NaI can be detected with 10 minutes incubation time (n=3).

Warning: This kit is only sold for the end users. Neither resale nor transfer to a third party is allowed without written permission from ABD Bioquest. Chemical analysis of kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@abdbioquest.com if you have any questions.