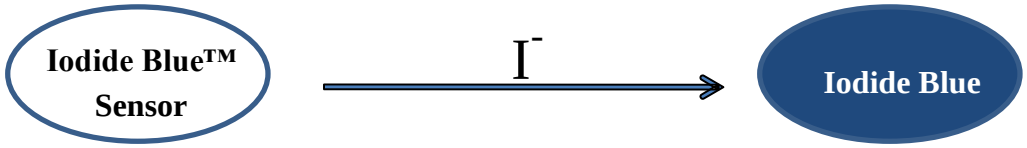
Screen Quest™ Colorimetric Chloride Channel Assay Kit

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 36350 (10 plates)	Keep at 4 °C Protect from 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 used to screen chloride channel modulators have to compromise between throughput, sensitivity and physiological relevance.

Screen Quest™ Colorimetric Chloride Channel Assay Kit provides a sensitive and robust colorimetric method for the study of chloride channels. The assay is based on our proprietary iodide indicator (Iodide Blue™) to measure iodide concentration, as low as 30 nM of iodide can be detected. Iodide Blue™ 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. Screen Quest™ Colorimetric 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.



Colorless

Yellow to Blue Color
(Signal can be read @380 nm, 405nm or 630 nm)

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

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:	Ease of use with less toxicity than the classic Sandell and Kolthoff assay.
Continuous:	Continuous assay without a separation step.

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 → Remove the growth medium → Add I^- loading buffer, treat cells with screening compounds → Wash cells with DPBS buffer 3 times → Lyse the cells with 1X lysis buffer (50 µL/well/96-well plate or 25 µL/well/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 secconds to 10 minutes→ Monitor 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 a 96-well plate or 10,000 to 20,000 cells/well/25µL 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 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/25µL 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:

2.1 Warm all the reagents to room temperature before use.

2.2 Make 1X I^- sensor enhancer solution: Add 50 µL of 100X Iodide sensor enhancer (Component B) 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.

2.3 Make 1X cell lysis buffer: Add the whole vial of 10X Cell Lysis Buffer (Component D) 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 ^oC.

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) and incubate for 2-4 hours.

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

3.4 Treat the cells with agonist in HBSS buffer for 5 minutes.
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 buffer.

3.5 Aspirate the supernatant.

3.6 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 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, and incubate for 5 minutes.

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

4.4 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 2.3)

4.5 Perform the iodide assay (See Step 5).

5. Run iodide assay:

5.1 Add 50 µL/well (96-well plate), or 25 µL/well (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) into the mixture (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 Monitor absorbance at 630, 380, or 405 nm.

Data Analysis

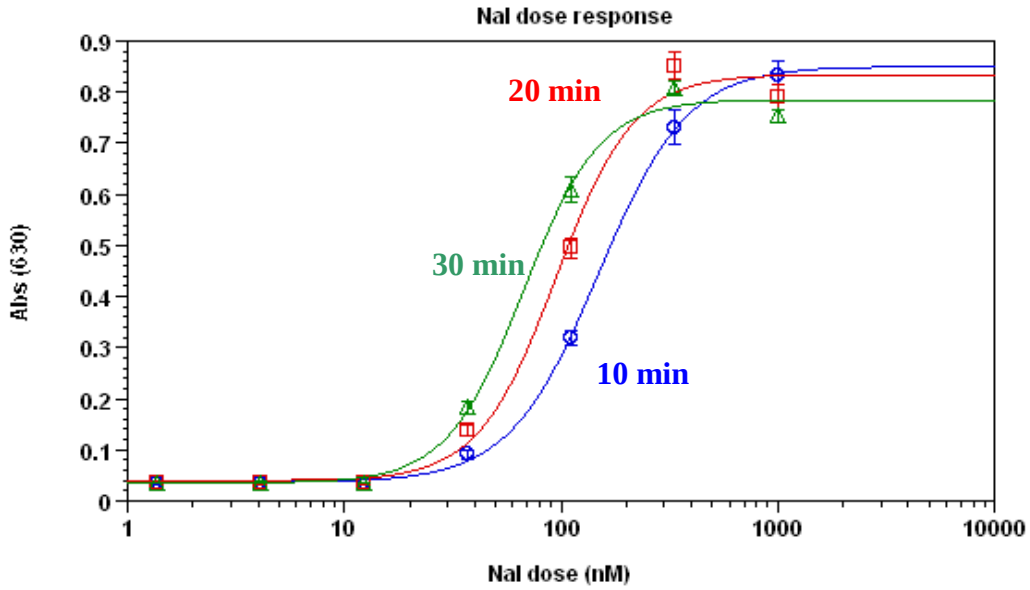


Figure 2. NaI dose response was measured with Screen Quest™ Chloride Channel Assay Kit on a black 96-well plate. As low as 30 nM of NaI was detected with 10 minutes incubation time (n=3).

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