Screen Quest™ Colorimetric Chloride Channel Assay Kit

Catalog number: 36350, 36351
Unit size: 10 Plates, 100 Plates

Component	Storage	Amount (Cat No. 36350)	Amount (Cat No. 36351)
Component A: Iodide Blue™ Sensor	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (50 mL)	1 bottle (50 mL)
Component B: Iodide Sensor Enhancer (100X)	Refrigerated (2-8 °C), Minimize light exposure	1 vial (0.5 mL)	1 vial (0.5 mL)
Component C: I- Loading Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (100 mL)	1 bottle (100 mL)
Component D: Cell Lysis Buffer (10X)	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (5 mL)	1 bottle (5 mL)

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. 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 was detected by this assay. Screen Quest™ Chloride Channel Assay Kit 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 and easily adapted to automation.

AT A GLANCE

Protocol Summary

1. Prepare cells
2. Remove the growth medium
3. Add I^- Loading Buffer, treat cells with screening compounds
4. Wash cells with DPBS buffer 3 times
5. Lyse the cells with 1X lysis buffer
6. Add equal volume of I^- sensor (50 or 25 µL)
7. Add 0.1X to 1X I^- Sensor Enhancer (50 or 25 µL)
8. Incubate at room temperature for 10 seconds to 10 minutes
9. Monitor absorbance at 380 nm, 405 nm, or 630 nm

Important Warm all the reagents to room temperature before use.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	630, 380, or 405 nm
Recommended plate	Clear bottom

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Cell Lysis Buffer (1X)
Add the whole vial of 10X Cell Lysis Buffer (Component D) to 45 mL of sterile H_2O and mix well.

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

PREPARATION OF WORKING SOLUTION

I^- Sensor Enhancer solution (1X)
Add 50 µL of 100X Iodide sensor enhancer (Component B) to 5 mL of sterile H_2O and mix well.

Note 1X I^- Sensor Enhancer solution is not stable; use within 2 hours after the dilution. Note: Each cell line should be evaluated on an individual basis to determine the optimal dilution of I^- Sensor Enhancer solution. We observed that 0.1X I^- Sensor Enhancer solution works even better for some cell lines.

SAMPLE EXPERIMENTAL PROTOCOL

For iodide efflux assay

1. Aspirate the growth medium from the cell plate.
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. Aspirate the Iodide Loading Buffer completely. Wash the cells with DPBS or HBSS at least 3 times.
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 or HBSS buffer.
5. Aspirate the supernatant.
6. Lyse the cells by adding 50 µL/well (96-well plate) or 25 µL/well (384-well plate) of 1X cell lysis buffer.

Perform the iodide assay.

For iodide influx assay

1. Aspirate the growth medium from the cell plate.
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.
3. Aspirate the Iodide Loading Buffer completely. Wash the cells with HBSS 3 times.
4. Lyse the cells by adding 50 µL/well (96-well plate) or 25 µL/well (384-well plate) of 1X cell lysis buffer.
5. Perform the iodide assay.

Run iodide assay

1. Add 50 µL/well (96-well plate) or 25 µL/well (384-well plate) of Iodide Blue™ sensor (Component A) to the wells from your choice of iodide influx/efflux assay.

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

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

3. Incubate at room temperature for 10 sec - 10 min.

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

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

4. Monitor absorbance at 630, 380, or 405 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

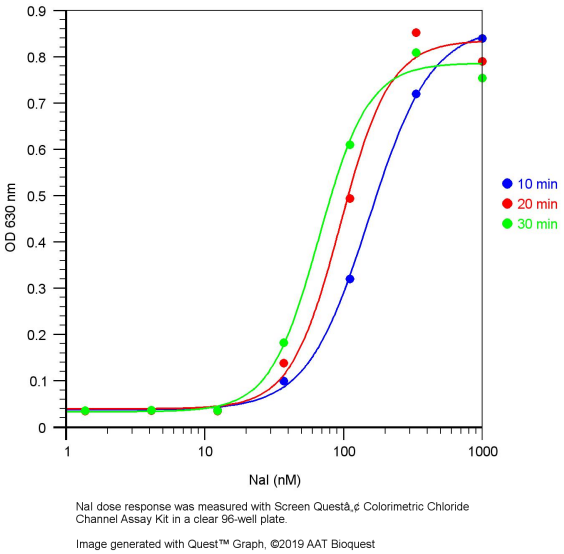

Figure 1. NaI dose response was measured with Screen Quest™ Colorimetric Chloride Channel Assay Kit in a clear 96-well plate.

DISCLAIMER

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