Premo™ Halide Sensor

Cat. no. P10229

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Premo™ Halide Sensor reagent (Component A)	20 mL	1X (ready to use)	• 2–6˚C • Protect from light • **Do not freeze**	When stored as directed the product is stable for 6 months.
BacMam enhancer (Component B)	1 vial	Not applicable	• 2–6˚C • **Do not freeze**	
Dimethylsulfoxide (DMSO, Component C)	1 vial	Not applicable	• ≤25˚C • Desiccate	
Premo™ Halide stimulus buffer	120 mL	2X	• 2–6˚C • **Do not freeze**	
Number of assays: Sufficient material is supplied for 10 × 96- or 384-well plates based on the protocol below.				
Approximate fluorescence excitation/emission maxima: Premo™ Halide sensor: 515/530 in nm.				

Introduction

Chloride channels are involved in a variety of important physiological functions that include transepithelial ion transport, cellular electrical excitability, and ion homeostasis.[1] Chloride channels represent valuable drug targets as a number of chronic disease states are due to defects in chloride channel function. The fluorescent protein based Premo™ Halide sensor from Invitrogen facilitates measurements of chloride channel activity in cells.

Premo™ sensors are delivered to mammalian cells using BacMam technology,[2-4] which employs baculovirus-mediated gene delivery (Figure 1). BacMam technology offers several advantages over other gene delivery methods: 1) high transduction efficiency across a broad range of cell types including primary and stem cells, 2) minimal microscopically observable cytopathic effects, 3) highly reproducible and controllable expression, 4) biosafety level 1 reagent as it will not replicate in mammalian cells and 5) simultaneous delivery of multiple genes. The Premo™ Halide sensor is supplied as a ready-to-use baculovirus stock and is used in conjunction with a BacMam enhancer for increased sensor expression enabling robust and reproducible analysis of chloride channel activity using high-throughput plate readers or fluorescence microscopy.

The Premo™ Halide sensor includes reagents for ten 96- or 384-well plates. Premo™ Halide sensor has been demonstrated to transduce multiple cell lines including BHK, U-2OS, HeLa, CHO, and primary human bronchial epithelial cells (HBEC) providing you the flexibility to assay chloride permeable channels in a wide range of cellular models.

Assay Principle

The Premo™ Halide sensor is based on the Venus variant of *Aequorea victoria* green fluorescent protein (GFP) which displays enhanced fluorescence, increased folding, and reduced maturation time.[5] The additional mutations H148Q and I152L were made within the Venus sequence to increase the sensitivity of the Venus fluorescent protein to changes in local halide concentration, in particular iodide ions.[6-8] The Premo™ Halide sensor exploits the sensitivity of the fluorescent protein to changes in halide concentration by employing iodide as a surrogate ion. Chloride channels are permeable to iodide (I) and therefore when a chloride channel opens iodide enters the cell due to the large gradient across plasma membrane. This channel opening is detected by a decrease (quench) of the Premo™ Halide sensor fluorescence (Figure 2). Premo™ Halide sensor shows a similar excitation and emission profile to yellow fluorescent protein (YFP) (Figure 3) and can be detected using standard GFP/FITC or YFP filter sets. Halide sensitive YFP-based constructs in combination with iodide quench have been used in high-throughput screening (HTS) to identify modulators of CFTR [9-12] GABA and glycine receptors, [13] and calcium activated chloride channels.[14]

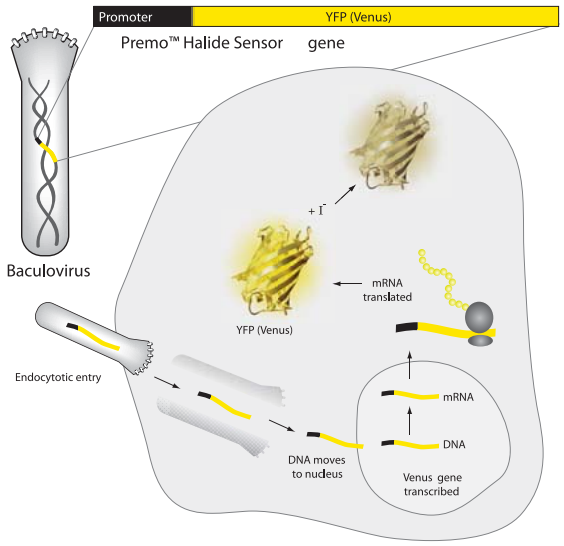

Figure 1. Schematic representation of Premo™ Halide sensor expression and halide sensitivity. Baculoviral particles encoding Premo™ Halide sensor enter the cells via an endocytotic pathway. Following cellular entry, the baculovirus moves to the nucleus where the Premo™ Halide sensor gene is expressed. The Premo™ Halide sensor protein is localized throughout the cytoplasm and is free to react with iodide ions upon chloride channel activation, resulting in a loss of fluorescence emission intensity.

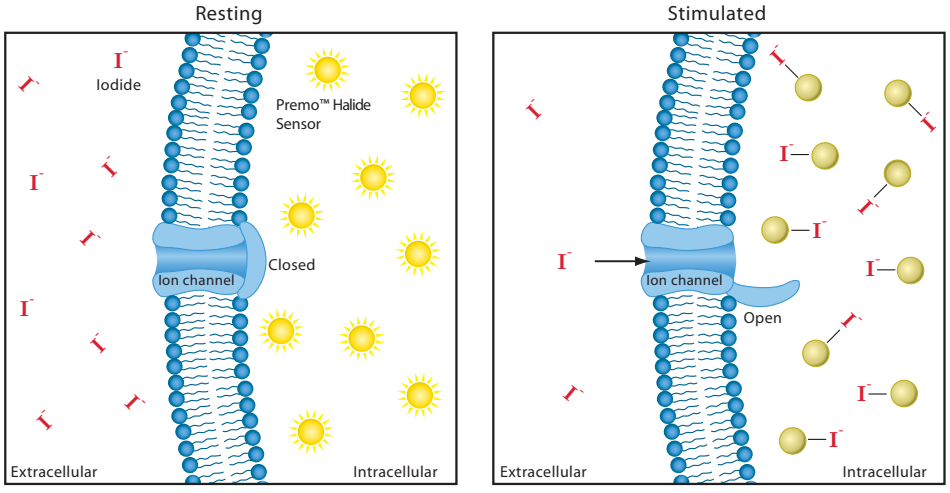

Figure 2. Principle of Premo™ Halide sensor: Iodide redistribution upon chloride channel activation. Basal fluorescence from Premo™ Halide sensor is high when chloride channels are low. Upon activation (opening) of chloride channels, the iodide ions enter the cell, down its concentration gradient, and quench the fluorescence from the Premo™ Halide sensor.

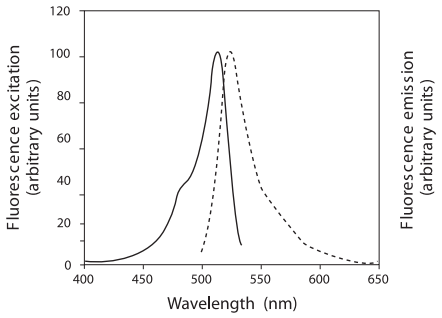

Figure 3. Fluorescence excitation and emission spectra of Premo™ Halide sensor.

Before You Begin

Materials Required but Not Supplied

Dulbecco's Buffered saline (D-PBS) without Ca^{++}/Mg^{++} (Invitrogen Cat. no. 14190-136)

Preparing Cultured Cells

Over 90 cell types have been successfully transduced using BacMam delivery technology including HeLa, CHO, BHK, SKBR3, HepG2, U-2OS, HEK 293, primary human bronchial epithelial cells, primary cardiac muscle cells, and NT2 human embryonal carcinoma cells. Currently, the delivery system does not work well for hematopoietic cells. For a complete list of cells compatible for use with Premo™ sensors, visit http://probes.invitrogen.com.

To use the Premo™ Halide sensor, plate ~1–4 × 10^6 mammalian cells/well in a 75 cm^2 culture dish. Allow cells to adhere and grow for approximately 4–24 hours at 37˚C and 5% CO_2 before proceeding with the transduction. Optimal transduction occurs when cells are at 70–80% confluency.

Alternatively, some cell types (U-2OS, 293) may be transduced while in suspension during passage for ease of use. See details below.

Preparing BacMam Enhancer Solution

Prepare the BacMam enhancer solution. Reconstitute the entire vial of enhancer (Component B) in 100 µL DMSO (Component C). Once reconstituted, the enhancer is a 1,000X solution. Store the reconstituted enhancer at 2–6ºC, protected from light

Note: The enhancer solution is stable to multiple freeze/thaw cycles following the recommended storage conditions. If desired, the solution can be divided into aliquots following reconstitution.

Preparing Premo™ Halide Stimulus Buffer

The Premo™ Halide stimulus buffer (Component D) is an iodide containing buffer composed of 150 mM NaI, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.4) and is supplied as a 2X concentrate. Equal volume of the 2X Premo™ halide stimulus buffer is added to an equal volume of cells in each well to achieve a final NaI concentration of 75 mM.

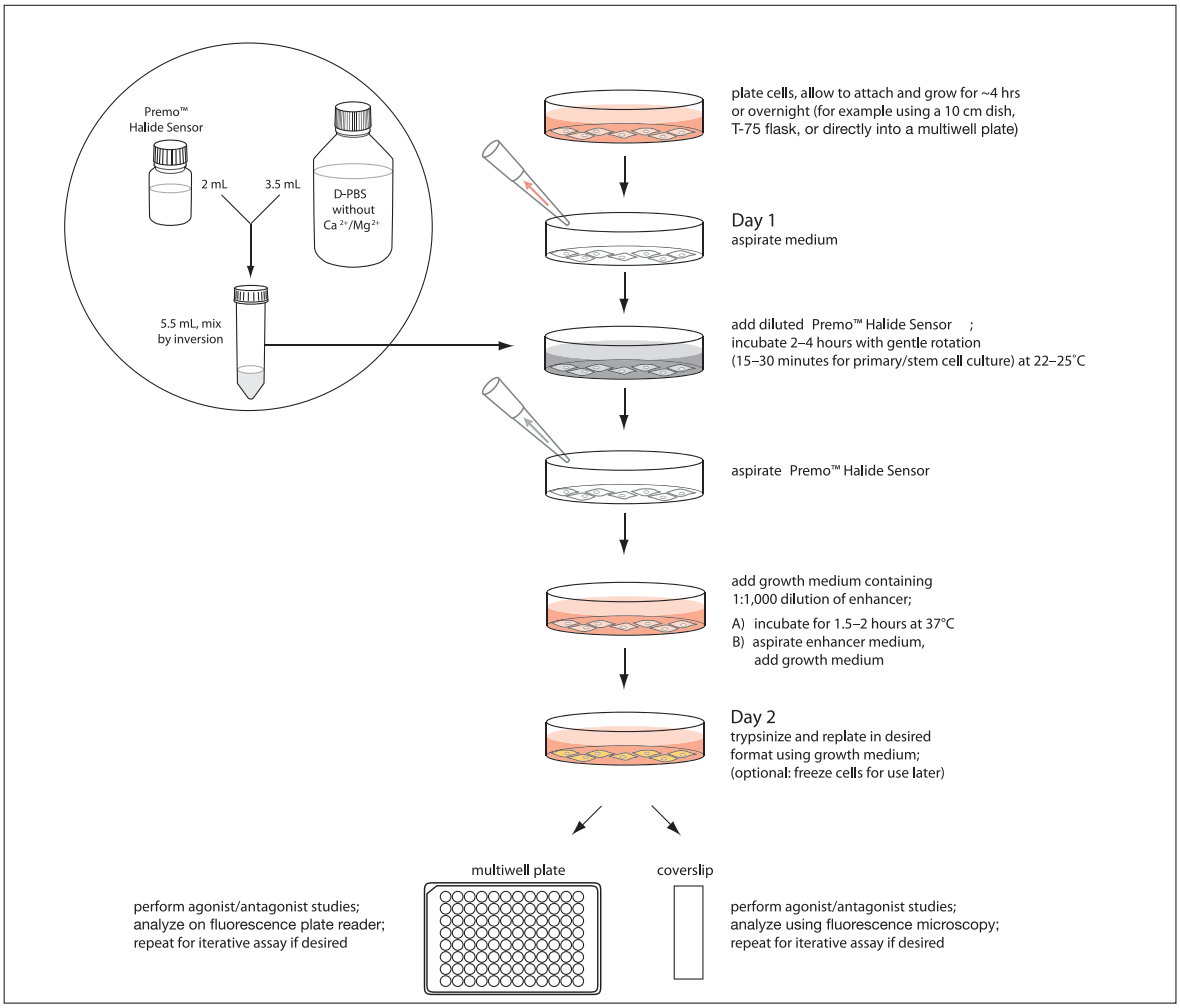

Figure 4. Schematic diagram of Premo™ Halide sensor workflow.

Sufficient buffer is supplied for ten microplates using 100 µL and 25 µL in 96- and 384-well formats, respectively.

Caution

- BacMam enhancer (Component B) may cause sensitization by skin contact, and is harmful by inhalation and if swallowed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable laboratory protective clothing and gloves while handling this reagent.
- DMSO (Component C) is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of reagents in compliance with all pertaining local regulations.

Transduction with Premo™ Halide Sensor

The transduction protocol with Premo™ Halide sensor described below was developed using adherent transformed cell lines and primary cell cultures. See Figure 4 for an overview of the transduction process. The transduction protocol can be modified for suspension cell culture as described below.

The reaction volumes are for a single 75 cm^2 cell culture dish. You may scale this protocol to other formats while maintaining the reagent/cell proportions. For optimal transduction efficiency, use low passage number cells at no more than 80% confluency.

Adherent Cells Workflow (New or Difficult Cells)

Day 1

Transducing Cells

1.1 Mix Premo™ Halide sensor reagent (Component A) by inversion to ensure homogeneity of the reagent prior to use.

1.2 Prepare 5.5 mL of Premo™ Halide sensor transduction solution in Dulbecco's Phosphate Buffered saline (D-PBS) without Ca^{++}/Mg^{++} (Invitrogen Cat. no. 14190-136) by combining 2 mL Premo™ Halide sensor reagent with 3.5 mL D-PBS.

Adjusting variables, such as the amount of Premo™ reagent and/or enhancer, incubation time, or cell density may help optimize expression levels to produce improved results. For example, optimize by varying virus to D-PBS ratio (e.g., use 0.5:4.5, 1:4, 1.5:3.5, 2:3, 2.5:2.5) at constant volume or increase the virus volume at constant D-PBS volume.

Note: Always protect the Premo™ Halide sensor transduction reagent (Component A) from light and place the vial back at 4˚C immediately after each use. Exposure to light over time will decrease the viral titer.

1.3 Aspirate cell culture media from adherent cells.

1.4 Add 5.5 mL Premo™ Halide sensor transduction solution (prepared in step 1.2) per 75 cm^2 dish or to every 4–6 × 10^6 cells.

1.5 Incubate the cells at **room temperature (20–25˚C)** for 2–4 hours with **gentle** rocking.

Note: Some cell types (i.e., primary and stem cells) are sensitive to a lack of calcium and magnesium and will begin to detach. Shorter incubation times (15–30 minutes) can be used with these cell types, though the transduction efficiency may be lower. BacMam reagent may also be added sequentially on a day-to-day basis to maintain expression levels or to obtain optimal expression in some cells types.

1.6 Aspirate Premo™ Halide sensor transduction solution from the cell culture dish.

1.7 Add appropriate cell culture media with or without serum with BacMam enhancer (i.e. 10 µL of prepared BacMam enhancer per 10 mL of media).

1.8 Incubate cells for 1.5–2 hours at 37˚C and 5% CO_2.

1.9 Aspirate enhancer medium and add normal growth medium without enhancer. Incubate cells at 37˚C and 5% CO_2 for >16 hours to allow expression of the Premo™ halide sensor.

1.10 Proceed to **Day 2, Plating Cells**, below.

Suspension Cells Workflow (HTS) Day 1 Transducing Cells

2.1 Harvest cells appropriately and resuspend cells in complete medium at the optimal density for plating in your assay.

2.2 Add Premo™ Halide sensor transduction solution (prepared in step 1.2) to the cells at 1–10% v/v.

2.3 Incubate cells at room temperature in the dark for 30–120 minutes with gentle rocking.

2.4 *Optional:* Add 1:1000 BacMam enhancer to the cells.

Note: This is an optional step and should be tested for each new cell type.

2.5 Dispense cells into microplates and incubate at 37°C and 5% CO_2 for >16 hours to allow expression of the Premo™ halide sensor.

2.6 Proceed to **Day 2, Plating Cells**, below.

Day 2 Plating Cells

3.1 **Optional:** At this stage, cells can be trypsinized or harvested and frozen in Recovery™ Cell Culture Freezing Medium (Invitrogen Cat. no. 12648-010) and stored in liquid nitrogen for future use

3.2 Harvest appropriately and plate in suitable assay plate (e.g., 96- or 384-well plate).

Note: Cells can be plated in complete medium with up to 10% serum. For optimal results, maintain cells near but below confluency at the time of the assay. Optimize the density of cells plated and the culture time for each system tested. When performing 96- or 384-well plate assays, we recommend including background wells which contain cells but without Premo™ halide sensor reagent.

3.3 If you are screening compounds, add and preincubate the compounds to be screened for 30 minutes prior to running the assay.

3.4 Cells are now ready for use with the chloride channel assay.

Note: In some cases, you may need to allow cells to settle or adhere to the bottom of a plate for few minutes to overnight before proceeding to the chloride channel HTS assay.

Chloride Channel HTS Assay for One Microplate of Cells

4.1 Measure the fluorescence intensity with a fluorescence plate reader using 510–520 nm excitation wavelength (standard YFP) or with standard green filters (480–500 nm). Detect emission between 520–560 nm. Instruments or microscopes that are filter based, require appropriate filters.

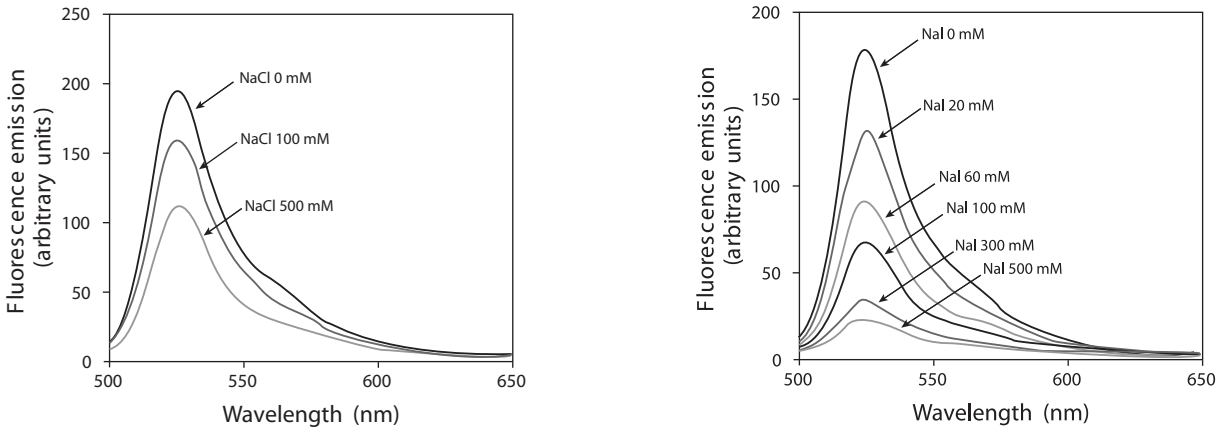

Figure 5. Quench of Premo™ Halide sensor fluorescence by increasing concentrations of iodide and chloride ions. U-2OS cells were transduced with Premo Halide™ sensor as described above. Cells were trypsinized 24 hours posttransduction and resuspended in sterile, distilled water to lyse cells. Total fluorescence quench at equilibrium was achieved by lysing cells and analyzing the fluorescence emission from the cell lysate. Fluorescence emission was examined using a fluorometer with increasing concentrations of NaCl (left panel) and NaI (right panel). Note the decrease in emission intensity with the increasing concentration of chloride and iodide ions. Iodide ions impose a greater quench of Premo Halide™ sensor fluorescence than chloride ions.

4.2 Typically, a chloride channel assay comprises of a base line reading (approximately 10 seconds) followed by a stimulation that activates the chloride channel population present in the assayed cell. The signal is typically followed for 60 seconds after stimulation.

4.3 Add an equal volume 2X Premo™ halide stimulus buffer containing NaI (Component D) to each well of the plate to obtain a final NaI concentration of 75 mM prior to addition of the chloride channel agonist.

Note: Since the plasma membrane of many cell types displays a limited permeability to iodide ions, provide a sufficient time window between the addition of NaI containing Premo™ Halide stimulus buffer and chloride channel agonist. This allows the passive redistribution of NaI across the plasma membrane through non-specific iodide entry pathways and avoids confounding the signal (quench) seen when the chloride channel of interest is activated.

Data Processing

The decrease (quench) of the fluorescence signal is indicative of activation of a chloride channel and the subsequent entry of the surrogate iodide ions, the duration and amplitude of the signal depends on the cell type, chloride channel expression level, and compounds used (Figure 5). Perform background subtraction by subtracting the background well values from the experimental wells. Data can be analyzed by taking the slope or peak of the iodide induced quench.

Note

The Premo™ Halide sensor assay relies upon the measurement of iodide ion flux across the plasma membrane through chloride channels with complex gating mechanisms. Effective concentrations of drug action obtained with this procedure may not reflect the results obtained by patch clamp studies; the Premo™ Halide sensor assay was developed as a screening, pharmacology, and hit-identification reagent.

References

1. Physiol Rev 82, 503 (2001); **2.** Nature Biotechnol 23, 567 (2005); **3.** Expert Opin. Drug Discov 2, 1669 (2007); **4.** Drug Disc Today 12, 396, (2007); **5.** Nature Biotech 20, 87 (2002); **6.** FEBS Letts 499, 220 (2001); **7.** Curr Biol 9, 17 (1999); **8.** J Biol Chem 275, 6047 (2000); **9.** Am J Physiol 281, C1734 (2001); **10.** J Biol Chem 277, 37235 (2002); **11.** J Biol Chem 278, 35079 (2003); **12.** Mol Pharmacol 68, 1736 (2005); **13.** Neurosci Lett 380, 340 (2005); **14.** Mol Pharmacol 73, 758 (2007).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
P10229	Premo™ Halide Sensor *for 10 microplates*	1 kit
12648-010	Recovery™-Cell Culture Freezing Medium	50 mL
14190-136	Dulbecco's Phosphate Buffered Saline (D-PBS) without Ca^{++}/Mg^{++}	1,000 mL

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probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

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