Cell Meter™ Fluorimetric Celluar Voltage Assay Kit

Catalog number: 35000
Unit size: 100 Tests

Component	Storage	Amount
Component A: VSB405	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: 100X Pluronic® F127	Freeze (< -15 °C)	1 vial (100 µL)
Component C: VSR555	Freeze (< -15 °C), Minimize light exposure	1 vial
Component D: Voltage Assay Buffer I	Freeze (< -15 °C)	1 bottle (50 mL)
Component E: Voltage Assay Buffer II	Freeze (< -15 °C), Minimize light exposure	1 bottle (10 mL)
Component F: DMSO	Refrigerated (2-8 °C)	1 vial (200 µL)

OVERVIEW

Almost all plasma membranes have an electrical potential across them, with the inside usually negative with respect to the outside. Signals are generated by opening or closing of ion channels at one point in the membrane, producing a local change in the membrane potential. This change in the electric field can be quickly affected by either adjacent or more distant ion channels in the membrane. Those ion channels can then open or close as a result of the potential change, reproducing the signal. Cell Meter™ Fluorimetric Celluar Voltage Assay Kit uses a FRET pair (VSB 405 and VSR 555) to monitor changes in cellular membrane potentials. The lipophilic VSB 405 is primarily located on the outer layer of lipid membranes while the localization of VSR 555 is sensitive to cellular membrane potential. At rest state, the inner layer of has a relatively negative potential, making VSR 555 predominantly located in close proximity to the outer layer of cell membranes, thus close to the blue fluorescent VSB405, resulting in efficient fluorescence transfer from blue (VSB 405) to red (VSR 555). When the cell is depolarized, VSR 555 translocates to the inner layer of the cell membrane, thus separating the FRET pair and disrupting FRET. The ratio of blue/red fluorescence is proportional to the cell voltage. This assay can used for screening compounds that modulate ion channels.

AT A GLANCE

Protocol summary

1. Grow cells as desired
2. Add VSB405 loading solution and incubate for 45 minutes at RT
3. Remove the VSB405 loading solution and wash cells with Voltage Assay Buffer I
4. Add VSR555 loading solution and incubate for 15 minutes at RT
5. Measure the response at Ex1/Em1 = 405/460 nm and Ex2/Em2 = 405/580 nm before and after addition of depolarizing stimulant

Important

Bring all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	405 nm
Emission	460 nm and 580 nm
Cutoff	435 nm and 515 nm
Recommended plate	Black wall/Clear bottom
Instrument specification(s)	Bottom read mode/Programmable liquid handling

Other instruments

FDSS, FLIPR, ViewLux, ArrayScan, FlexStation, IN Cell Analyzer

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.

1. VSB405 stock solution (100X)
Add 100 µL DMSO (Component F) into VSB405 (Component A) and mix well.

Note Store the unused VSB405 stock solution at -20 °C in single use aliquots.

Note VSB405 stock solution is stable for at least one month at indicated storage conditions.

2. VSR555 stock solution (100X)
Add 100 µL DMSO (Component F) into VSR555 (Component C) and mix well.

Note Store the unused VSR555 stock solution at -20 °C in single use aliquots.

Note VSR555 stock solution is stable for at least one month at indicated storage conditions.

PREPARATION OF WORKING SOLUTION

1. VSB405 loading solution
Add 10 µL 100X Pluronic® F127 (Component B) into 1 mL of Voltage Assay Buffer I (Component D), mix well. Add 10 µL VSB405 stock solution and mix well.

Note VSB405 loading solution should not be stored and should be used promptly.

2. VSR555 loading solution
Add 10 µL VSR555 stock solution into 1 mL of Voltage Assay Buffer II (Component E) and mix well.

Note VSR555 loading solution should not be stored and should be used promptly.

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol can be used as a guideline and should be optimized according to the needs.

1. Grow cells as desired.
2. Remove the cell culture medium and add 100 µL Voltage Assay Buffer I (Component D).
3. Immediately remove the Voltage Assay Buffer I.

   Note It is not necessary to incubate or shack the cells with Voltage Assay Buffer I.
4. Add 100 µL VSB405 loading solution to each well and incubate at room temperature for 45 minutes, with plate covered and protected from light.
5. Remove VSB405 loading solution and wash cells with 100 µL Voltage Assay Buffer I. Remove Voltage Assay Buffer I.
6. Add 100 µL VSR555 loading solution to each well and incubate at room temperature for 15 minutes, with plate covered and protected

from light.

7. Read cells in plate reader at Ex1/Em1= 405/460 nm and Ex2/Em2= 405/580 nm. Plate reader will take readings in resting potential and then inject depolarization buffer such as high K buffer, before taking several more readings.

   Note Test compounds should be added immediately after loading the cells with VSR555. Stimulants should be added while acquiring data.

EXAMPLE DATA ANALYSIS AND FIGURES

Calculate the Average 460 initial and 580 initial and 460 final and 580 final values

1. Take the initial fluorescence intensity measurements (Polarized state) at 460 and 580 nm wavelengths before adding depolarizing stimulant.
2. Add depolarizing stimulant and continue to record fluorescence intensity readings until changes in fluorescence intensity measurements (depolarized state) have reached a plateau.
3. Select identical data windows (as shown in boxes in the figure) for both initial and final readings at 460 and 580 nm.
4. Calculate the average of the data points within a data window to obtain Average 460 initial, 580 initial, 460 final and 580 final values.

Calculating the baseline corrected values

1. Obtain the baseline signals as above from control wells. Control wells are wells without cells and select data points same as earlier.
2. Calculate the average of all the four data windows to obtain baseline 460 initial, 580 initial, 460 final and 580 final values.
3. Calculate the baseline corrected values by subtracting the appropriate average baseline values from the average data values for each data window.

Calculating emission ratios

Calculate the emission ratio for depolarized and polarized states as shown below:

Emission ratio (Polarized) = 460 initial / 580 initial (Use background corrected values)

Emission ratio (Depolarized) = 460 final / 580 final (Use background corrected values)

Determining the response ratio

Response Ratio = Emission ratio (Depolarized) / Emission ratio (Polarized)

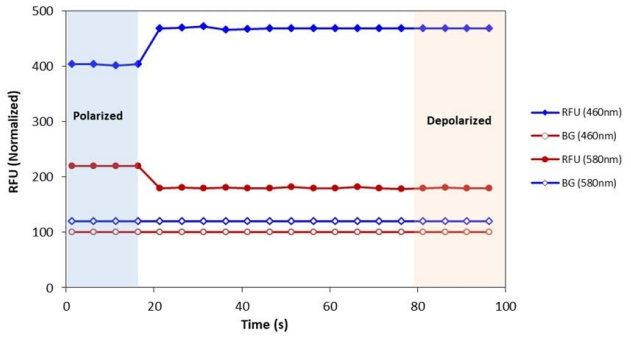

Figure 1. The monitoring of membrane potential in HeLa cells using Cell Meter™ Fluorimetric Cellular Voltage Assay kit. HeLa cells were stained according to the kit instructions, and stimulated with depolarizing solution (164.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, 20 mM HEPES, pH 7.4). The response was recorded using FlexStation 3 (Molecular devices).

DISCLAIMER

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