FluoVolt™ Membrane Potential Kit

Catalog Number F10488

Pub. No. MAN0009668 Rev. D.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Changes in membrane potential play a central role in many physiological processes, including nerve-impulse propagation, muscle contraction, and cell signaling. Potentiometric probes are important tools for studying these processes and are generally characterized as slow- or fast-response probes.

Slow-response probes function by entering depolarized cells, binding to proteins or membranes, and exhibiting enhanced fluorescence. This membrane translocation event decreases the ability of these reporters to respond to rapid changes in membrane potential and introduces a capacitive load, which can affect cell health. However, slow response probes display a high magnitude of response; typically in the 1% per millivolt (mV) range.

Molecules that change their structure in response to the surrounding electric field can function as fast-response probes to detect transient (millisecond) potential changes. However, when compared to the slow-response probes, the magnitude of potential-dependent fluorescence change of the fast-response probes is often small (2–10% fluorescence change per 100 mV).

The FluoVolt™ membrane potential dye represents the next generation in voltage-sensitive probes and brings together the best characteristics of the fast- and slow-response membrane potential probes with sub-millisecond membrane potential changes and a greater response.

For easy cell loading, the FluoVolt™ Membrane Potential Kit contains the PowerLoad™ Concentrate solution. Due to its unique nature, the PowerLoad™ Concentrate solution can be used in the presence of complete culture medium, reducing the negative effects of replacing the medium or loading in serum-free medium.

The Neuro Background Suppressor solution greatly reduces the amount of baseline autofluorescence caused by growth medium components.This solution is specially formulated to minimize osmotic shock and is not harmful to neuronal cells. In addition, Neuro Background Suppressor solution has been used successfully with many different cell types to efficiently suppress background fluorescence without sacrificing the specific cellular fluorescence generated in the assay.

Contents and storage

Sufficient material is supplied for 25 assays based on the protocol below.

Approximate fluorescence excitation and emission maxima: Use standard FITC or GFP filter sets for visualization and imaging.

Item	Amount	Storage[1]
FluoVolt™ Dye, 1,000X in DMSO (Component A)	50 µL	• 2°C to 8°C • Dessicated • Protected from light • DO NOT FREEZE
PowerLoad™ Concentrate, 100X (Component B)	500 µL	• 2°C to 8°C • DO NOT FREEZE
Neuro Background Suppressor, 10X (Component C)	5 mL	• 2°C to 8°C • Protected from light • DO NOT FREEZE

[1] When stored as directed, product is stable for at least 1 year.

Required materials not provided

- Cell line and culture medium of choice
- Live Cell Imaging Solution (LCIS) (Cat. No. A14291DJ), (physiological buffered saline equivalent to Ringer's solution)
- Buffered and pH-adjusted physiological saline solution for dye loading and imaging for long-term studies. Depending on the cell type, phosphate-buffered saline (PBS), Hank's balanced salt solution (HBSS), Ringer's solution, or Krebs' solution can be used
- Sterile-filtered 2 M Glucose Stock Solution for use with LCIS to support cell health in long-term (hours) experiments using primary or differentiated neural cells
- Glass-bottom culture dishes or coverslips

Prepare working LCIS solution with 20 mM glucose

Dilute 2 M Glucose Stock Solution 1:100 into LCIS for a final glucose concentration of 20 mM.

Note: After adding glucose, prevent the growth of bacteria, fungi, and yeast to ensure the solution remains free of contaminants.

Load cells with FluoVolt™ membrane potential dye

The protocol below provides instructions for performing the membrane potential assay using cells grown in a 35-mm dish with 2 mL of culture medium.

1. Prepare fresh FluoVolt™ Loading Solution by adding 100 µL of 100X PowerLoad™ concentrate (Component B) and 10 µL of 1,000X FluoVolt™ dye (Component A) to a 15-mL tube, then vortex to mix.
2. Add 10 mL of physiological buffer of choice or working LCIS solution, then invert the tube to mix.
3. Remove medium from adherent cells and wash cells twice in physiological buffer of choice or LCIS.
4. Add 2 mL of FluoVolt™ Loading Solution to cells, and incubate cells at room temperature for 15–30 minutes.
5. Remove FluoVolt™ Loading Solution-and wash cells twice in physiological buffer of choice or LCIS.
6. Add 2 mL of physiological buffer of choice or 20 mM Glucose Stock in LCIS. Cells are now ready for live-cell imaging.
7. *(Optional)* To suppress background fluorescence, add Neuro Backdrop Background Suppressor solution diluted 1:10 (Component C).

Image cells loaded with FluoVolt™ dye

Standard FITC settings can be used to visualize the membrane staining of FluoVolt™ dye. Short exposures (10 ms or less) are possible with 2 × 2 or greater pixel binning, but depends on hardware configurations to measure rapid or successive depolarizations. To ensure positive responses from the dye, treat cells with 10 µM Valinomycin (a potassium ionophore, Cat. No. V1644) for 30 minutes, then add an equal volume of isotonic potassium chloride (KCl) solution to depolarize the cells.

Note: Isotonic KCl is composed of 140 mM KCl, 5 mM NaCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 20 mM HEPES, 20 mM glucose, NaOH (pH 7.4).

Related products

Product	Amount	Cat. No.
FluoVolt™ Membrane Potential Kit	1 kit	F10488
Live Cell Imaging Solution	500 mL	A14291DJ
Valinomycin	25 mg	V1644
Hanks' Balanced Salt Solution (HBSS) (1X), liquid	500 mL	14025-092
HEPES Buffer Solution (1 M)	20 mL	15630-106

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0009668 D.0

Revision	Date	Description
D.0	8 December 2023	Updated the storage temperature.
C.0	7 May 2021	Updated the protocol by removing the following: "Optional: Add 100 µL of 100X Probenecidstock solution to prevent extrusion of cytosolic dye by anion pumps, which can decreaseloading efficiency on some cell types." Transferred to the CCMS.
B.0	16 February 2018	Revised the filter sets required for visualization and imaging, reformat, update regulatory/legal boilerplate language.
A.0	30 October 2013	New document for FluoVolt™ Membrane Potential Kit.