molecular probes®
by life technologies™

FluoVolt™ Membrane Potential Kit

Catalog no. F10488

Table 1 Contents and storage

Material	Amount	Storage	Stability
FluoVolt™, 1000X in DMSO (Component A)	50 µL	• 2–8°C • Dessicate • Protect from light • DO NOT FREEZE	When stored as directed the product is stable for at least 1 year.
PowerLoad™ Concentrate, 100X (Component B)	500 µL	• 2–8°C • DO NOT FREEZE	
Neuro Background Suppressor, 10X (Component C)	5 mL	• 2–8°C • Protect from light • DO NOT FREEZE	

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

Approximate fluorescence excitation and emission maxima: Absorption and fluorescence spectra of the FluoVolt™ dye is highly dependent on the environment. Standard FITC filter sets should be used for visualization and imaging.

Introduction

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 response 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 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: it responds to changes in membrane potential in sub-milliseconds and displays a high magnitude of response.

For Research Use Only. Not for use in diagnostic procedures.

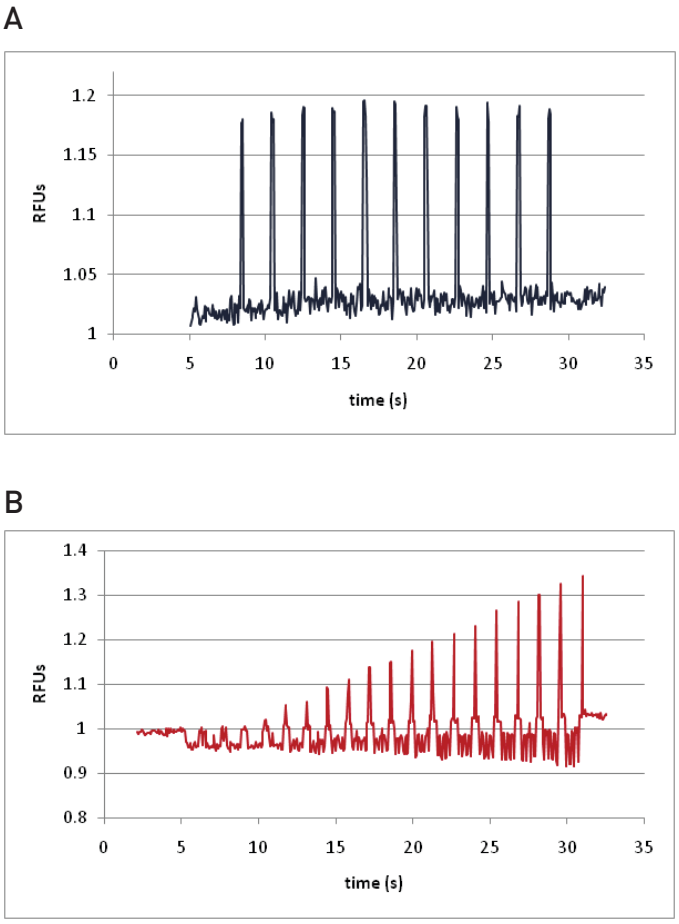
For easy cell loading, the FluoVolt™ Membrane Potential Kit contains the PowerLoad™ Concentrate solution. Due to its unique nature, the PowerLoad™ solution can be used in the presence of complete culture medium, thus reducing the negative effects of replacing the medium or loading in serum-free medium.

Baseline autofluorescence caused by components within the growth medium can be greatly reduced by the addition of the Neuro Background Suppressor solution. The Neuro Background Suppressor solution has been specifically formulated for use with neuronal cells and will not cause osmotic shock. Additionally, the 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.

Properties

- Fast – responds to changes in membrane potential within sub-milliseconds
- High Sensitivity – response range is up to 25% per 100 mV
- Emission/excitation (522/535 nm) works with standard FITC settings
- Can be used in imaging or patch clamp applications

Figure 1 Patch clamp analysis of human HEK 293 cells were loaded with FluoVolt™ membrane potential dye. Cells were imaged with 10 millisecond illumination pulses and images were acquired with 2X binning. Traces show fluorogenic responses as cells are depolarized for 100 milliseconds **(A)** at 2 second intervals from –100 mV to +30 mV, or **(B)** in single steps from –80 mV to 0 mV at 2 second intervals.



Before You Begin

Materials required but not provided

- Cell line and culture medium of choice
- For optimal performance, we recommend Live Cell Imaging Solution (LCIS) (Cat. no A14291DJ), a physiological buffered saline equivalent to Ringer's Solution.
- Buffered and pH adjusted physiological saline solution for dye loading and imaging. Phosphate buffered saline (PBS), Hank's balanced salt solution (HBSS), Ringer's solution, or Krebs' solution are acceptable depending on cell type, and should be pH or osmotically adjusted for signaling or long term studies.
- A sterile-filtered, 2 M Glucose Stock Solution will also be required for LCIS and many other solution formats to support cell health in longer term (hours) experiments, as well as studies with primary or differentiated neural cell types.
- For optimal performace, we recommend glass-bottom culture dishes or coverslips.

Prepare 20 mM Glucose + Live Cell Imaging Solution

Dilute 2 M Glucose Stock Solution 1:100 into LCIS for a final glucose concentration of 20 mM. Keep this solution clean and free of contaminants to prevent bacterial, fungal, or yeast growth once glucose has been added.

Experimental Protocols

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.

1.1 To a 15-mL tube, add the following reagents in the order listed below to prepare fresh FluoVolt™ Loading Solution:

100X PowerLoad™ concentrate (Component B)	100 µL
FluoVolt™ dye, 1000X (Component A)	10 µL

Vortex to mix.

Physiological buffer of choice or 20 mM Glucose Stock in LCIS	10 mL

Invert the tube to mix.

Optional: Add 100 µL of 100X Probenecid stock solution to prevent extrusion of cytosolic dye by anion pumps, which can decrease loading efficiency on some cell types.

1.2 Remove medium from adherent cells and wash cells twice in physiological buffer of choice or LCIS.

1.3 Add 2 mL of FluoVolt™ Loading Solution (from step 1.1) to cells, and incubate cells at room temperature for 15–30 minutes.

1.4 Remove FluoVolt™ Loading Solution, and wash cells twice in physiological buffer of choice or LCIS.

1.5 Add 2 mL of physiological buffer of choice or 20 mM Glucose Stock in LCIS. Cells are now ready for live-cell imaging.

Optional: To suppress background fluorescence, add 1:10 diluted Neuro Backdrop Background Suppressor solution (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 milliseconds or less) are possible with pixel 2 × 2 binning or greater, but will depend on hardware configurations to measure rapid or successive depolarizations. To confirm positive responses from the dye, treat cells with 10 µM Valinomycin (a potassium ionophore, Cat. no. V1644) for 30 minutes, and 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, pH 7.4 NaOH.

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

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

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These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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