FDSS7000EX FDSS/μCELL

Direct comparison of Fast response and Slow response Membrane potential dye using the FDSS6000

Abstract

Ion transport proteins including channels, transporters, and pumps play central roles in cellular bioenergetics, excitability, and a multitude of other biological functions. Major approaches for ion channel drug screening include voltage clamp electrophysiology, radioactive flux or tracer assays, binding assays with whole cell or membrane preparations, and cellular fluorescent ion and membrane potential indicators. The use of cellular fluorescent probes is another important method that is used for drug discovery and development, especially for primary screening where high throughput assays are often required. Here we discuss cell-compatible fluorescent probes, functional assays, and FDSS instrumentation that are used to monitor real-time target activity and screen large chemical libraries for potent and selective modulators. High throughput screening (HTS) compatible, rapid kinetic assays are emphasized, in particular the use of slow-response and fast-response voltage-sensitive membrane potential probes to assay ion channel activity in single cells and 96/384-well formats.

Materials and Methods

Fluorescent dye and Optical requirements (filters)

Anionic dyes such as $DiBAC_4(3)$ are more responsive to plasma membrane potential. The ANEP (aminonaphthylethenylpyridinium) dyes developed by Leslie Loew and colleagues are the most consistently sensitive probes for detection of submillisecond membrane potential changes. DiBAC excites at 488 nm and emits in the 510 nm-570 nm range, and has typically been used at 2 μM to 5 μM final concentration. Di-8-ANEPPS excites at 488 nm and emits in the 620 nm-660 nm range, and has typically been used at 5 μM to 10 μM final concentration For FDSS6000 applications, the excitation and emission light are separated with a dichroic mirror, we recommend a 490-540 nm long-pass dichroic with the steep onset of transmission.

Loading conditions

HEK cell transfeced by HERG channel receptor were tripsinized and plated in 96 or 384-well plate. Dye loading concentrations vary with different cell types and must be empirically determined for the given cell type. Optimal loading conditions generally range between 5-10 μM final dye concentration. A typical buffer soluion is Hank balance salt solution with 10 mM HEPES and 2 g/L glucose added. The pH of the buffer solution shoud be adjusted to pH 7.2-7.4. After staining, the excess dye is removed by washing the extracellular solution with serum-free buffer. The stock solution is diluted with of a serum-free buffer with vortexing.

Results and Discussion

Many kind of voltage sensitive probes are used to screen ion channels in mammalian cells. The most commonly used intensity dye is $DiBAC_4(3)$. This is the negatively charged oxonol dye that equilibrates between the extracellular solution and cellular sites according to the cellular membrane potential.

Di-8-ANEPPS has a fairly uniform about 10 % per 100 mV change in fluorescence intensity in a variety of tissue, cell and model membrane systems ANEP dyes undergo changes in their electronic structure, and consequently their fluorescence spectra, in response to changes in the surrounding electric field. This optical response is sufficiently fast to detect transient potential changes in excitable cells, including single neurons, cardiac cells and intact tissue preparations. Furthermore, these dyes display a potential-dependent shift in their excitation spectra, thus permitting the quantitation of membrane potential using excitation ratio measurements (Ex440 and 530 nm Em>570 nm or Ex.480 nm Em.620 nm/560 nm). Structural variations among the ANEP dyes confer suitability for specialized applications.

Results and Discussion

We have compared the effects of KCl on HERG K(+) channels stably expressed in HEK 293 cells loaded by both dyes. On addition of several concentration KCl, the kinetics of fluorescence changes of $DiBAC_4(3)$ and Di-8-ANEPPS dye using FDSS6000 was identified. As expected, fluorescent ratios were increased immediately after stimulation of KCl (Figure.1). We have varidated the first data using the voltage-sensitive slow- fast-response dye with the FDSS6000. Thus this Voltage-sensitive membrane potential dye represents a powerful tool for developing high-throughput screening assays for ion channels.

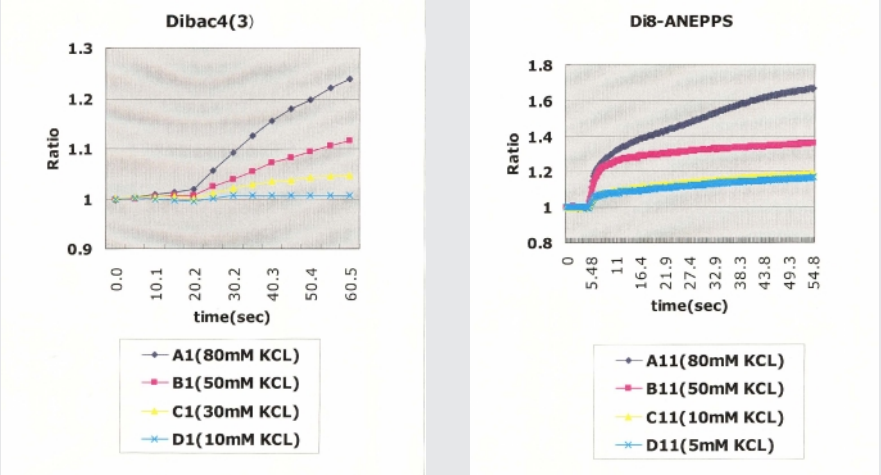

Fig. 1: HEK cells were transfected with HERG channel vector. Above signal is the change in fluorescence due to addition of KCl. Enlargement of 60 seconds of recording.The change in fluorescence due to addition of KCl at T = 60 seconds

Consumable

Catalog Number B-438
Product Name bis-(1,3-dibutylbarbituric acid)trimethine oxonol ($DiBAC_4(3)$)

Catalog Number D-3167
Product Name di-8-ANEPPS

References

1. Fluhler E, Burnham VG, Loew LM
Spectra, membrane binding, and potentiometric responses of new charge shift probes.
Biochemistry. 1985 Oct 8;24(21):5749-55.

2. Jing Zhang, Robert M, Davidson, Mei-de Wei, and Leslie M. Loew
Membrane Electric Properties by Combined Patch Clamp and Fluorescence Ratio Imaging in Single Neurons
Biophysical Journal Volume 74 January 1998 48-53

3. A. Bullen and P. Saggau
High-Speed, Random-Access Fluorescence Microscopy: II. Fast Quantitative Measurements With Voltage-Sensitive Dyes
Biophysical Journal Volume 76 April 1999 2272-2287

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