Reagents for Determining Cellular Membrane Potentials

Ordering Information	*Storage Conditions*
Product Numbers: 21410 (25 mg), 21411 (25 mg), 21412 (5 mg), 21414 (25 mg)	Store at -20 °C, desiccated and protected from light

Introduction

DiBAC4(3), DiBAC4(5) and DiSBAC2(3) are a set of sensitive slow-response membrane potential probes that are widely used for measuring membrane potentials of many biological systems. In general, slow-response probes exhibit potential-dependent changes in their transmembrane distribution that are accompanied by a large fluorescence change. The magnitude of their optical responses is much greater than that of fast-response probes (typically a 1% fluorescence change per mV). Slow-response probes, which include cationic carbocyanines, rhodamines and anionic oxonols, are suitable for detecting changes in average membrane potentials of nonexcitable cells caused by respiratory activity, ion-channel permeability, drug binding and other factors.

Chemical and Physical Properties

Membrane Potential Indicators	Catalog Numbers	Excitation	Emission	Molecular Weight	Solvent
DiBAC4(3) [Bis-(1,3-dibutylbarbituric acid)trimethine oxonol]	21411	493 nm	516 nm	516.64	DMSO
DiBAC4(5) [Bis-(1,3-dibutylbarbituric acid)pentamethine oxonol]	21410	590 nm	616 nm	542.67	DMSO
DiSBAC30111	21412	535 nm	560 nm	366.42	DMSO
DiSBAC2(3) [Bis-(1,3-diethylthiobarbituric acid)trimethine oxonol]	21414	535 nm	560 nm	436.55	DMSO

Assay Protocol with Membrane Potential (MP) Indicators (Example of DiSBAC2(3))

Brief Summary

Prepare cells in growth medium → Add dye-loading solution (100 µL/well for a 96-well plate or 25 µL/well for a 384-well plate) → Incubate at RT or 37 °C for 30 min to 1 hour → Read fluorescence intensity at Ex/Em = 490/525 nm (Cat. # 21411), 590/625 nm (Cat. # 21410) or 540/590 nm (Cat. # 21414)

Note: Following is our recommended protocol for live cells. It only provides a guideline, and should be modified according to your specific needs.

1. Prepare cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 40,000 to 80,000 cells/well/100 µL for 96-well plates or 10,000 to 20,000 cells/well/25 µL for 384-well plates.

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellets in equal amount of HHBS and MP dye-loading solution (see Step 2.2 below) at 125,000 to 250,000 cells/well/100 µL for 96-well poly-D lysine plates or 30,000 to 60,000 cells/well/25 µL for 384-well poly-D lysine plates. Centrifuge the plates at 800 rpm for 2 minutes with brake off before the experiments.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for the intracellular calcium mobilization.

2. Prepare DiSBAC2(3) dye-loading solution (for 1 plate):

2.1 Prepare a 10 to 30 mM stock solution of DiSBAC2(3) in high-quality, anhydrous DMSO. The stock solution should be used promptly; any remaining solution need be aliquoted and frozen at ≤ -20 °C.
Note: Avoid repeated freeze-thaw cycles, and protect from light.

2.2 Prepare a 2X DiSBAC2(3) dye-loading solution: On the day of the experiment, either dissolve DiSBAC2(3) solid in DMSO or thaw an aliquot of the DiSBAC2(3) stock solution to room temperature. Prepare a 2X working solution of 20 to 40 µM in Hanks and 20 mM Hepes buffer (HHBS) or buffer of your choice, pH 7 with 0.04% to 0.08% Pluronic® F-127 (Cat. # 20053) and 2 mM Trypan Red Plus™ (Cat. # 2456). Mix them well by votexing. This working solution is stable for at least 2 hours at room temperature.

3. Run Membrane Potential Assay:

3.1 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) DiSBAC2(3) dye-loading solution (from Step 2.2) into the cell plate.
Note1: If your screen compounds interfere with growth medium and serum factors, replace the growth medium with equal volume of HHBS buffer before adding the DiSBAC2(3) dye-loading solution. Alternatively, cells can be grown in serum-free conditions.
Note 2: Do NOT wash the cells after dye loading.

3.2 Incubate the dye-loading plate in a cell incubator for 30 to 60 minutes.
Note: In some cases, incubation at room temperature for 30 to 60 min may work better.

3.3 Prepare the compound plates by using HHBS or your desired buffer.

3.4 Run the membrane potential assay by monitoring the fluorescence intensity at Ex/Em = 540/590 nm (Cat. # 21414).
Note: It is important to run the signal test before your experiment. Different instruments have their own intensity range. Adjust the signal test intensity to the level of 10% to 15% of the maximum instrument intensity counts. For example, the maximum fluorescence intensity count for FLIPR-384 is 65,000, so the instrument settings should be adjusted to have its signal test intensity around 7,000 to 10,000.

Data Analysis

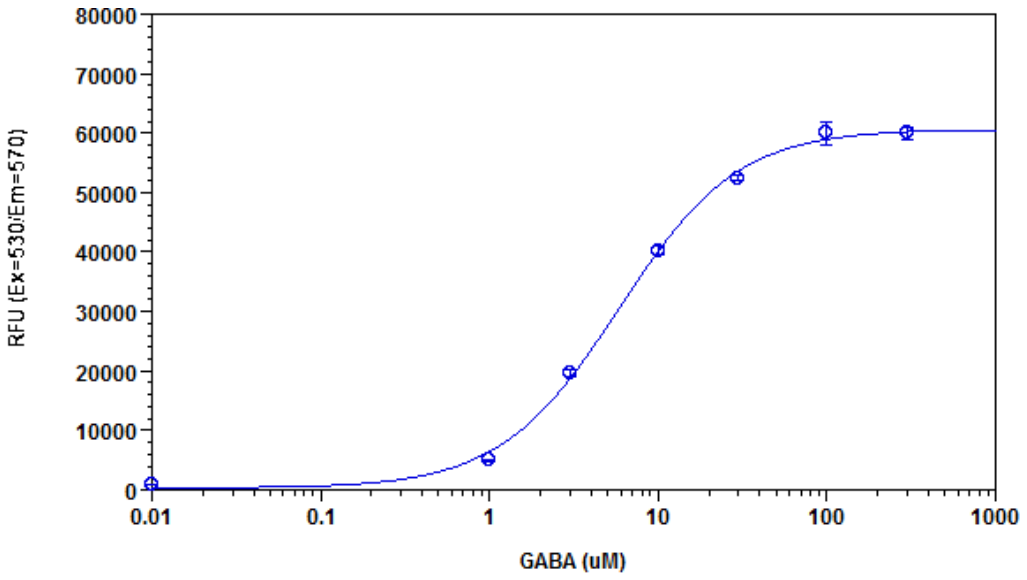

Figure 1. GABA Dose Response was measured with DiSBAC2(3) in WSS-1 cells**.** WSS-1 cells were seeded overnight at 50,000 cells/100 µL/well in a 96-well black wall/clear bottom costar plate. The cells were incubated with 100 µL of DiSBAC2(3) dye loading solution for 30 minutes at room temperature. GABA (50 µL/well) was added by FlexStation (Molecular Devices) to achieve the final indicated concentrations.

Disclaimer: *This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact our technical service representative for more information.*