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Membrane Depolarization Assay Using DiBAC4

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[1]In-house protocol

1 Works for me This protocol is published without a DOI.

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ABSTRACT

MD assay protocol (signup for the flow machine for the date and time you want to run)
Membrane Depolarization Assay Using DiBAC4(3) (Bis(1,3-Dibutylbarbituric Acid)TrimethineOxonol)

PROTOCOL CITATION

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GUIDELINES

- DiBAC4(3)
- Bacterial cells
- Staining
- Using flow-cytometer(LSR II Becton Dickinson -488 nm for DiBAC4 assay)
- Cleaning after use
- Analyzing data

DISCLAIMER:

ABSTRACT

MD assay protocol (signup for the flow machine for the date and time you want to run)

Membrane Depolarization Assay Using DiBAC4(3) (Bis(1,3-Dibutylbarbituric Acid)TrimethineOxonol)

BEFORE STARTING

DiBAC4(3)

- Thermo Fischer, B438 – 25 mg. Mix with 1 ml of DMSO (stock solution 25mg/ml); Prepare a 100 ug/ml working solution in distilled water.
- Required concentration 1ug/mL, use 5 ul of DiBAC4(3) working solution per 500 ul.

Bacterial cells

- Harvest your cells in FACS tubes. Based on the OD of the cells the volume of harvest may vary, eg. OD ~ 0.3 – 100ul; OD ~ 1.0 – 25uL.

Staining

1 Wash the cells in ~ 4 mL of Phosphate Buffer Saline (PBS), 3000 RPM -10 minutes

2 Resuspend in 0.5 mL of PBS, add 5 ul of DiBAC4(3) working solution

3 Mix (vortex gently) and incubate in dark at room temperature for 20 minutes

4 Wash the cells twice with PBS, and in chem hood resuspend in 100 ul of formaldehyde (2% in PBS).

5 Mix and incubate in dark for 10 minutes in the hood. Centrifuge for 5 minutes and discard the supernatant in the appropriate disposal container

6 Wash with 1 mL of PBS twice, resuspend in 0.5mL PBS. Mix well and analyze in a flow cytometer.

Using flow-cytometry (LSR II Becton Dickson -488nm for DiBAC4 assay)

7 Before turning on the machine check the volume of sheath fluid (fill if not sufficient) and waste (empty if more than half).

8 The probe of the machine must be in the tube with MiliQ water. Turn ON the machine. It should be on **LOW** and **STANDBY** mode. Press **RUN**.

9 In the computer Log in- Fozo (Fozo123)

10 Clicking View button- bring the panels to Desktop (Acquisition Dashboard etc.)

11 Establish a new setup OR duplicate existing setting without Data. Enter all your sample labels. (I keep my samples in order as the sample labels entered so that its easy to run)

12 Set total events to be run to 100,000.

13 Move the sliding arm beneath the tube. Remove the tube (with water) and place your sample. Click **acquire data** and adjust the events per second to **~3000/second**. If low press medium or high button on the flow; if high dilute the sample by adding PBS.

14 Once the events/second is stable ~ 3000/second, press record data. This will record 100,000 events.

15 Click "**Next sample**" OR the sample you want to run, and replace the tube with next sample.

16 Analyze all of your samples. Once complete make sure you clean the machine thoroughly.

17 Export the data to the external drive. (click Time- "None")

Cleaning after use

18 After you run your last sample, have a tube with 10% bleach (about 3/4th filled) and run this in "Hi" leaving the arm on the side (not beneath the tube) for 1 minutes. Keep an eye on the volume in tube (it decreases rapidly). If necessary, take the tube out and add bleach. Move the arm beneath the tube with bleach and let it run "Hi" for 5 minutes

19 Remove the tube with bleach and repeat the process with the cleaning detergent, followed by water.

20 After the final run of the water (for ~ 7 mins) , press "Low" and then "Standby". Turn off the machine.

Analyzing data

21 Install FlowJo in your computer. Take FlowJO drive from Sparer lab and open your exported file in FlowJo.

22 Click one of your samples, you will see a figure showing the distribution of the cells, click **Histogram** on the Y-axis and **FITC** on the X-axis. Set your cut off to separate cluster of cells (I use 10^3 abu of fluorescence- cells with higher fluorescence intensity will be designated as depolarized).

23 This will generate sub-population of the cells. Drag one of this sub-population to the file you imported. This will generate sub-populations for all your samples.

24 Export the excel file of this data. If you want to have the figure of the histogram use layout to construct representative histogram from your samples.

25 You can analyze your data from the excel file OR use prism.