**Staining of yeast cells with DHE**

Last update on 2012 June 4, Hong Qin

**Background**

Dihydroethidium, DHE, is a fluorescent probe arguably for superoxide anions (O2\*-).

**Procedure**

1. Grow yeast cells in a choice of media and desired length of period.
   1. For log-phase, it is safe to restage the culture in desired media on the same day.
2. Spin down 1ml of cells, wash with 1ml PBS, and resuspend in 1ml PBS
3. Make master mix of DHE stain solution
   1. 190 ul PBS + 1ul of 5mM DHE stock (for 10ul of cells)
   2. Use 5mM DHE stock, (Dihydroethidium or hydroethidine, 5 mM stabilized Solution in DMSO, Invitrogen D-23107, $195.98)
   3. Final concentration of DHE is **25uM of DHE** .
4. Label reaction tubes with strain, treatment, and reaction time (if necessary)
5. Aliquot 10 ul of cells from the 1ml suspension to reaction tubes, and add 191ul of DHE mix
6. 30C incubation in dark for 10 minutes.
   1. For time series, back-calculate the incubation length, so all the sample will finish incubation at the same time.
7. Spindown, resuspend in 1ml PBS, keep them in boxes wrapped with aluminum foils.
8. Proceed to FACS analysis

**BD FACS Calibur usage**

**Caliburation of BD FACS Calibur using CaliBRITE Beads**

1. Check sheath tank and waste tank. Add sheath and empty waste is necessary.
2. Switch fluidics to pressurized.
3. Turn on BD FACS Calibur machine
4. Turn on computer and login.
5. While the Calibur is warming up (ususaly for at least 15 minutes?), prepare the beads.
6. Prepare two 12x75mm Falcon polystyrence tubes, label them as TubeA and TbueB
7. Add 1ml of sheath fluid to tubeA
8. Add 3 ml of sheath fluid to tube B (If PerCP-Cy5.5 is used, BD recommends bead dilution buffer should be used. However, we have ignored this)
9. Gently mix the CaliBRITE beads
10. For 3 color calibration, add 1 drop of Unlabaled beads to A; 1 drop of Unlabled, FITC, PE and PerCP each.
11. Keep these prepared beads suspensions ice and shield from light.
12. Run FACSComp
13. … …
14. setup to finish the calibration.

**Measurement**

1. Open CellQuest
2. Connect to Calibur
3. Usually signals are best viewed in log-scale. (The data are still saved in normal scale)
4. Make sure that P1, P2, P3, etc are saved with channel names.
5. Use unlabeled samples to find the baselines and to set appropriate threshold to eliminate debris.

**Notes:**

\* Paul Doetsch lab label yeast cells with DHE in YPD because they found cells metabolize well in YPD. (If cell mainly metabolized on endogenous carbonhydrates, this should not be a problem).

\* Fran Madeo label DHE with 10 minutes of incubation at 30C.

\* In BD FACSCalibur, I found DHE can be detected in FL2 (585/42nm) and FL3 (680LP), but not in FL1 (530/30nm) (see note on 2012 Feb 22).

**References:**

DHR can be measured in FL1 (530nm+/15nm) in FACSCaliber2. DHR is green

H2DCF-DA, green fluorescence can be collected at 525nm band pass in Beckman’s EPICS XL-MCL. (FITC channel).

In **Mesquita** PNAS paper, DHE signals can be captured by FL3 (>670nm) in FACSCaliber2. So, this is red. Intracellular superoxide anions were measured using dihydroethidium (DHE) (Molecular Probes). Aliquots of cells were collected at indicated time points and DHE was added to a final concentration of 5 μM from a 5-mMstock in DMSO. After incubation for 10 min at 30 °C, cells were washed once with 0.5 mL PBS, resuspended in 50 μL PBS, and added to 1 mL PBS. After

briefly sonicating the suspension, DHE signals were measured

using a FACSCaliber2 flow cytometer (BD-Biosciences) with

a 488-nm excitation laser. Signals from 25,000 cells/sample were

captured in FL3 (>670 nm) at a flow rate of 5,000 cells/s. FACS

measurements of DHR signals presented in Fig. 2 were measured

similar to the DHR measurements described for Fig. 1 except that

a FACSCaliber flow cytometer was used to capture signals in FL1

(530 nm ± 15 nm) from 25,000 cells/sample at a flow rate of 5,000

cells/s. Data collected with the FACSCaliber2 flow cytometer were

processed with Flowjo software (Tree Star) and quantified with

WinList software (Verity Software House)

Q FL4 calibration