Live/Dead assay by FACS Analysis

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Make an appointment online:
http://Hviflow.duhs.duke.edu/calender
Username: CARL232; Password: hviflow
Minimum sign up time is 30 min, 24 hour notice by phone for cancellation
(Rates: $57.86/h for phenotype; $80.32/h for sorting; $28.93/h for consulting)

Procedures:

1. Seed cells in 6-well plates ($1x10^5$ cells/well) one day before transfection.
2. Transfection with 0.25 μg of the gene of interest in expression vector (i.e. pCI-neo or Igf1r-Myc), 0.25 μg refernce maker gene (i.e. pGFP-N2) and 2 μl of lipfectamine 2000/well. Change medium to 10% FBS/DMEM 6 hours later.
3. Check the transfection efficiency under microscope by observing GFP+ cells at about 24 hrs after transfection. Change medium with or without freshly prepared 100 μM H_2O_2 and incubate for another 24 hours
4. Harvest the medium including the floating cells into conical tubes on ice. Trypsinize the remaining adherent (~100 μl 0.25% Trypsin-EDTA solution) cells and wash with 1 ml 10% FBS/DMEM twice. Pool the cells and spin at 2000 rpm (The Beckman centrifuge in the culture room) at 4°C for 10 min.
5. Wash cells with 1 ml ice-cold PBS followed by 1000 x g spin (The desktop enpendorff centrifuge) for 10 min at 4°C.
6. Suspend the cells in 500 μl PBS, put them on ice and bring to FACS facility on campus.
7. Add PI (1 μg/ml) and incubate at room temperature for 10 min. Load the cells for FACS

Note: