

## FACS Protocol

1. Harvest 0.5ml of 1.0 OD<sub>600</sub> cells or 1 ml of 0.5 OD cells.
2. Resuspend in 0.5 ml of 1XPBS
3. Add 1ml of 100% EtOH
4. rotate a minimum of 1hr at RT or O/N at 4<sup>0</sup>C (max 1 week)
5. Pellet and wash with 1ml of 50mM Tris pH7.5
6. Pellet and add 1mg/ml RNase in 10 mM Tris pH 7.5 and vortex
7. Incubate at 37<sup>0</sup>C for 1-2 hours, or 4<sup>0</sup>C overnight
8. Add 2µl of 10mg/ml Proteinase K
9. Vortex and incubate for an hour at 50-55<sup>0</sup>C (temperature, time is critical)
10. Pellet and wash with 1 ml of 1X PBS
11. Sonicate for 5 Seconds with 30% duty cycle.
12. Pellet and resuspend the cells in the dark with 200ul of 100ug/ml propidium iodide [in PBS and azide].
13. Incubate for 1 hour - days
14. Dilute 200 µl to 1 ml in 1XPBS.