Cell Cycle - DNA Content

Fixation

1. Liberate cells from tissue culture flask by trypsin digestion and collect in a 15 ml conical tube.
2. Collect cells by centrifugation 3000xg 4 min and resuspend in 1 ml PBS.
3. Repeat PBS wash.
4. Add 3 ml 70% ethanol (-20ºC) slowly DROP-WISE directly from the freezer while vortexing VERY GENTLY (No. 2 vortex setting on mixer).
5. Incubate on ice for 30 min
6. Collect cells by centrifugation 3000xg 2 min and resuspend in 1 ml Flow Buffer or PBS.
7. Adjust volume to adjust the cell concentration.
8. Store tightly capped at 4ºC.

Staining

1. At least 30 min before analysis begins stain the cells.
2. Combine 0.1 ml of cells with 0.9 ml stain solution.
3. Incubate 30 min (or longer) at room temperature in the dark.
4. Stained samples may be stored in the dark at 4oC.

Staining Solution

10ml		
Stock Propidium Iodide	(4.5 mg/ml)	33 ul
RNase A	(20 mg/ml)	400 ul
Water		9.57 ml

Reference For This Method

You J, Bird RC. Selective induction of cell cycle regulatory genes cdk1 (p34cdc2), cyclins A/B, and the tumor suppressor gene Rb in transformed cells by okadaic acid.J Cell Physiol. 1995 Aug;164(2):424-33.