Preparing cells for PI/FACS (cell cycle) analysis

Experimental design considerations

- This method works well to assess cell cycle distribution of whole cell populations
- This method can also be used to assess the cell cycle distribution of GFP transfected cells however, the EtOH step is generally not sufficient to keep GFP in the cell. The use of membrane localized GFP fusion vectors [i.e. Us9-GFP described by Kalejta et al, Exp. Cell Res. 248; 322 (1999) or GFP-SP described by Kalejta et al, Cytometry 29;286 (1997)] provides excellent results employing this EtOH fixing procedure and is highly recommended to assess the cell cycle distribution in the transfected cell population.

Protocol

- Harvest cells (typically from one 10 cm plate)
- Wash 1X with 5-10 ml of 1X PBS
- Suspend cells in 500ul 1X PBS containing +0.1% Glucose (keep at 4°C)
- Immediately add 5 ml of cold 70% ETOH (keep at –20°C)
- Mix immediately
- Keep at 4°C for between 1hr to 1wk to fix cells

- Two to three hours prior to FACS analysis
    - spin cells down
    - wash 1X with 1X PBS (10ml)
    - without adding more PBS, spin again for 2 min so that the residual PBS can be taken off
    - after taking off remaining PBS, suspend in 300ul propidium iodide solution (keep at 4°C)
    - add 20ul of 10mg/ml RNase
    - mix and incubate at 37°C for 30-45min.
    - transfer to FACS tubes and have 'em analyzed.

Buffer

<u>Propidium Iodide Solution</u>
- 69uM Propidium Iodide (Sigma, Cat#P4170)
in 38mM NaCitrate (pH 7.4)