

DNA Staining For Flow Cytometry

Method

Collect cells for staining and spin 1200rpm 10 min 1200rpm 10min 4°C, and wash in twice in PBS by centrifugation.

Resuspend the cells in 10ml of ice cold 80% ethanol (store at -20°C) rapidly, and leave to fix overnight. Cells will be fine at this stage for months, but will "swell"!!

Centrifuge the cells at 1200rpm 10min 4°C and wash in PBS (cells will "contract")(NB you can transfer to an ependorf tube, and spin at room temperature for 5min at 4500rpm in a microfuge if you have a small pellet of cells after the first PBS wash).

Resuspend the cells in PBS with 0.1% Triton-X100 and 0.1mM EDTA and add 50µg/ml of propidium Iodide (2.5µl of 20µg/ml) and incubate at 4°C overnight.

Strain the cells though a FACS cap tube, or a cell strainer.

Run on FACS.

Reagents

80% ethanol store at -20°C for at least an hour before use.

Propidium Iodide 20mg/ml solution Fluka 81845 **NB TREAT THIS SOLUTION IN THE SAME WAY AS ETHIDIUM BROMIDE!!!!.**

RNase only use DNase free RNase, ie boiled stock.