

2.3.1.4 Differentiation of U937 Cells

Before western blot and flow cytometry experiments U937 cells were differentiated. Cells were re-suspended in culture medium containing PMA (150 nM) then left to differentiate for 24 h to give an adherent macrophage-like phenotype (Picone et al., 1989).

2.3.1.5 Cell Counting

For the adherent cell lines the cells were washed, trypsinised, and re-suspended in a known volume of medium to form a cell suspension as described above. From this cell suspension, an aliquot (100 μ l) was removed and diluted by half with a trypan blue solution in PBS (100 μ l; 0.2 % w/v), mixed and allowed to stand for 1 min to stain dead cells. A coverslip was placed onto the haemocytometer slide, and following the appearance of Newton's rings, the counting chamber was filled with the cell suspension. A total of eight (0.1 mm³) squares were counted. To count viable cells, any non-viable cells stained with trypan blue were excluded. An average of the counts was made and this equates to the number of cells $\times 10^4$ per ml, this is doubled to account for the trypan blue dilution to give the number of cells/ml in the suspension i.e. average of 8 squares $\times 2 = \text{cells/ml} \times 10^4$.

In the case of the suspension cells (U937) cell counting was undertaken as described above with the exception that cells were removed from the flask, centrifuged at 200 RCF, and re-suspended in a known volume before placing on the haemocytometer slide.

2.3.1.6 Cell Freezing

Cells were regularly frozen to maintain stocks. At any time at least two frozen vials were stored in liquid N₂. To freeze cells, a cell suspension was first counted to give numbers of viable cells, re-centrifuged, and the resulting pellet was then re-suspended in cold FBS containing 10 % DMSO with a pipette to give 1×10^6 cells/ml. Then 1 ml of this cell suspension was added to a cryogenic vial, wrapped in tissue paper and placed in a polystyrene box which was maintained at -20 °C for 1 h, then at -80 °C overnight. Finally the vial was placed in liquid N₂. Slow freezing is essential as it helps to preserve the cells because ice crystals (that can be produced by rapid freezing) are less likely to form, and rupture the cells.