

(test performed using the polymerase chain reaction technique by Kerri Winship, Welsh School of Pharmacy, Cardiff University, UK).

### ***2.3.1.1 Thawing of Cryopreserved Cells***

Cells were obtained from the supplier as a cryopreserved cell suspension. They were rapidly thawed in a 37 °C water bath and then placed in a universal container containing 5 ml of complete medium (Table 2.2) and centrifuged at 200 RCF for 5 min to remove the cryopreservative (DMSO). After centrifugation, the supernatant was decanted and replaced with fresh medium containing 10 % v/v FBS (5 ml). The cell pellet was gently re-suspended using a pipette. The resulting cell suspension was placed in a flask (25 cm<sup>2</sup>) and allowed to grow for 24 h. Cells were then washed once with PBS and supplemented with complete medium (Table 2.2) and maintained as described below.

### ***2.3.1.2 Maintenance of Adherent Cell Lines (COS-1, COS-7, MCF-7, Caco-2, DU145 and PC3)***

Cells were maintained in 75 cm<sup>2</sup> vented tissue culture flasks in the appropriate media (Table 2.2). Cells were subcultured weekly i.e. when they were 70-90 % confluent. First the cell culture medium was removed from the flask using a sterile quill then cells were washed with phosphate buffered saline (10 ml, PBS 0.1 M; pH 7.4) before the cells were trypsinised (1 ml trypsin/EDTA <3 min incubation at 37 °C). The flask was then tapped to free adhered cells, and 10 ml of culture medium used to wash the cells off the side of the flask, the resultant cell suspension was transferred to a universal container and centrifuged at 200 RCF for 5 min. The supernatant was removed and the cells re-suspended in 5 ml of culture medium with a 23 gauge needle and syringe. The resulting cell suspension was then used to subculture the cells at their appropriate split ratio (Table 2.2). All cell lines were used for a maximum of 30 passages before culturing a new batch of cells. This ensured that the cells were always within the same passage range for all studies.

### ***2.3.1.3 Maintenance of Cells in Suspension***

U937 cells were subcultured 2-3 times weekly i.e. when they were at 70-90 % of their maximum cell density. Cells were harvested by centrifugation at 200 RCF for 5 min. Supernatant was removed and the pellet was re-suspended in culture medium with a 10 ml pipette and used to subculture the cells at the appropriate ratio (Table 2.2). Again, U937 cells were kept for a maximum of 30 passages before culturing a new batch.