

Matteck Corporation, USA). This microscope was also used to acquire bright-field images of cells.

2.2.2 General Equipment

A Toledo 320 pH meter from Mettler (Toledo, Switzerland), 3150 pH meter (Jenway) or Hydrion pH indicator paper (Sigma-Aldrich; UK) were used for pH measurements.

Samples were freeze-dried with a Flexi Dry FD-1.540 freeze-dryer from FTS Systems (USA) connected to a DD75 double stage, high vacuum pump from Javac (Australia).

SDS-Page gels were cast in a Bio-Rad vertical gel cast and the electrophoresis current supplied by Bio-Rad Power Pac 300 (Bio-Rad, UK). Agarose gels were prepared in a 11x14 cm gel cast and the subsequent electrophoresis was performed in a Horizon 11.14 electrophoresis tank from Life Technologies, Gibco (UK) with current supplied by EC135 Transformer (EC Apparatus Corp., USA).

Centrifugation was performed with a Varifuge 3.0 RS centrifuge supplied by Heraeus Instruments (Germany). When speeds greater than 4000 relative centrifugal force (RCF) were needed an Optima LE-80K centrifuge from Beckman Coulter (USA) was used. Eppendorfs were centrifuged in an Eppendorf 5417 R bench-top centrifuge with standard fixed angle rotor (model 5417 C/R; Eppendorf, Germany).

2.3 General Methods

Here, those general methods are described which were used throughout the studies repeated in more than one of the following Chapters. Specific methods integral to the substance of the specific studies e.g. synthetic methods are repeated in the relevant Chapter.

2.3.1 Cell Culture

Cell culture was carried out according to the guidelines provided by ATCC (ATCC, 2003). All manipulations with the exception of the centrifugation step, were performed in a class II laminar flow hood using sterilised consumables that had first been decontaminated with 70 % (v/v) ethanol. Cells were incubated and maintained in incubators at 37 °C in a 5 % CO₂ atmosphere that had a water humidifying tray containing copper II sulphate unless otherwise stated. All cells were mycoplasma free

(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 cm²) 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 cm² 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.