Apoptosis assay with MDCK cells

MDCK cells grown in low serum will die by apoptosis which can be assayed by isolating low molecular weight DNA from the cells by the Hirt lysis method and running it on an agarose gel to visualize the degradation of chromatin into nucleosome length fragments (nucleosome ladder). Cells are plated out into 60mm dishes with 5ml medium containing 10%, 0.5% and 0.1% serum.

To set up the cells:

make up 100ml medium for each serum concentration.

1. Trypsinize cells, remove from the dish in 5ml complete medium (medium + 10% serum + antibiotics). Count the cells.
2. For the 0.5% and 0.1% serum samples aliquot 4 x 106 cells into sterile 15ml conical tubes. Aliquot 1 x 106 cells for the 10% serum samples.
3. Spin down cells at #2.5, 5min in the benchtop centrifuge. Wash the cells with 5ml PBS and spin again.
4. Resuspend each cell pellet in 5ml of the required serum concentration and dispense into a 60mm dish. Incubate 48 hours, 37oC.

To harvest the cells:

1. Scrape off the cells with the medium (there may be cells floating and you want them to) into 15ml conical tubes. Spin down at # 2.5 in the benchtop centrifuge.
2. Resuspend the cell pellet in 1ml PBS, transfer to a microfuge tube. Spin down gently (1500rpm or pulse for 2 secs).
3. Remove all except ÷ 50ul of the PBS, loosen the pellet by flicking the tube and immediately add 400ul Hirt Lysis buffer. Invert the tube several times to mix DO NOT VORTEX, do not put on ice.
4. Add 100ul 5M NaCl, invert 2 - 3 times to mix, place the tubes, upside down, at -20oC. Leave for 1 to 2 hours.
5. Thaw the samples at room temperature, spin down high molecular weight DNA 15mins at +4oC.
6. Remove the supernatent, extract with an equal volume of TE saturated phenol and precipitate with 2.5 volumes ethanol.
7. Spin down the DNA, wash the pellet with 70% ethanol, air dry.
8. Dissolve the pellets in 20ul TE + 20ug/ml boiled RNase A, incubate at room temp for 30mins. Add 5ul 5 X Orange G loading dye and run on a 1.2% agarose/TAE gel.