Cell cycle analysis:

Plate cells according to time points. Confluency=30%

After 24 hrs of culture in complete media, serum starve the cells. (Medium-DMEM+7.5% BSA)

To synchronize G0/G1 phase, let the cells grow for 48-72 hrs.

After the set time, harvest the cells at different time points (intervals of 6h, 12h, 18….) and fix in chilled 70% ethanol.

Fixation:

Trypsinize the cells and wash with PBS. Transfer cells to flow tubes in 100uL PBS.

While vortexing, add chilled (-20 C) 70% ethanol dropwise (~2 mL) to avoid clumping. This can be stored at -20 C for 2-3 weeks.

Annexin V and PI staining:

After harvesting, spin down by washing with PBS containing 2% FBS and 1% HEPES for 10 mins at 2000 rpm.

Discard the supernatant and dissolve the pellet in 400 uL PBS (2% FBS and 1% HEPES). Pass this through 40um cell strainer.

To this add 15 ul annexin V and incubate for 1 hr at 37 C. Wash with cold PBS buffer once. Resuspend in 200 ul PBS buffer.

Add PI (25 ug/ml) to the same tube and keep in dark at 37 C for 30 mins.

Store at 4 C covered in foil until flow acquisition.