dsRNA transfection (serum starvation protocol)

Materials:
6-well plates
15 ml Corning conical tubes

*Use cells that have been split the day before.

1.) Harvest cells as above.
2.) Count cells.
3.) For a 6-well plate, seed 1 million cells in each well. Determine # of cells needed for each experiment and aliquot as appropriate.
4.) Spin cells down @ 1000 rpm for 3 min in 15 ml polypropylene tube.
5.) Aspirate supernatant.
6.) Resuspend cells to 10^6 cells/ml in Serum Free Media (SFM).
7.) Aliquot 1 ml of cell suspension into each well of a 6 well plate.
8.) Add the RNA. For initial time course experiment, use 20 ug of DIAP1 RNA. A titration of DIAP1 dsRNA will be done to determine the ideal amount of RNA to add to the cells.
9.) Serum starve cells by leaving cells in SFM for 1 Hour.
10.) Add back 2 ml of normal media (15% serum, so that final [serum] is 10%.
11.) Analyze the cells for initial DIAP1 experiment at 24 hours, 48 hours and 72 hours after transfection and determine the amount of cell death as assayed by trypan blue staining.

Notes

• Speed number 4 on the floor centrifuge in the tissue culture room corresponds to 1000 RPM.