

To Load Cells On to Gradient

Add cells into C. Open F. Open B. When B is opened, it isn't closed again until gradient is prepared.
Add the 80 mls cell suspension, then clamp at F when all cells are out of C.
Wash-the small chamber C X 2 with MEM (O) to remove trace cells.
Add MEM (5) up to black mark (level with the 940 mls of other 2 chambers).

The gradient is now ready to begin. Start magnetic stirrers in C & D.

Take off clamp fit H, then G, and finally F.

Gradient will start to run.

It will take about an hour to run. (preferably less)

When all MEM (FCS) is in chamber I, switch off magnetic stirrers and tap B.

Gradient sits for 12 hrs. from this time.

Next morning

Take 50 X 30 mls aliquots using tube J.
Spin 7' @1400 rpm cold into 2mls 3% MEM count-decide on factious-pulse
Height analyzer
Pool decided factious, spin, resuspend to give $35-50 \times 10^6$ cells/nat.