**Protocol for Colony Forming Unit (CFU) (1000 cells/mL)- 7 days**

**Day 0**

1. Thaw Methylcellulose a day before experiment. Put in 4C fridge. Otherwise in RT on that day of experiment.
2. Get ready all items: 6 well plates (for duplicate), 1ml syringes, special wide-bore needle, white tubes with transparent cap (bacti type).
3. Add in 1% Pen/Strep into the stock Methycellulose. Vortex and mix.
4. Aliquot 4mL Methylcellulose into each labelled white tubes.
5. Prepare ML2, KI ML2, dstomato cell lines. Centrifuge and withdraw supernatant. Resuspend in 1mL RPMI. Take 10uL + 90uL HBSS to count cell.
6. Further dilute the cell to get 0.2M/mL. Final amount to get is 1000cell/mL. Eg.since we need 4mL in the tubes, thus we need 4000cells/4mL.

\*Eg 33uL +970uL HBSS

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| --- | --- | --- | --- |
| ML2 | 90/9 | 0.2M/ml | 20uL |
| Ds | 104/9 | 0.23M/ml | 17.4uL |
| 246 | 86/4 | 0.19M/ml | 21.5uL |
| 259 | 97/9 | 0.22M/ml | 18.1uL |

1. Prepare Stock for PLKi 1uM and 5uM.(Final conc will be 1nM & 5nM). Pipette in 4uL of PLK4i into each labelled white tubes. Vortex to mix (updown)-> Fast spindown using the centrifuge. Press”Fast”
2. Prepare 1mL syringe and the special black needle.

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| --- | --- | --- |
| 1nM Well 1 | Well 2 | Well 3 |
| 5nM Well4 | Well 5 | Well 6 |

1. Pipette in 1mL into each 3 well
2. Take 200uL pipette tip to remove bubble.
3. ##Prepare additional 1 6 well plates for staining.

**Day 3@4**

1. Check under microscope the cells in well.
2. Prepare a new 12 well plate (eg. for 4 cell line with 0,1nM, &5nM dose PLK4i)
3. Mix the cells in the well before seed out.
4. Prepare 800uL RPMI in each new well upfront.
5. Dilute to 1:5 (200uL ML2 cell to 800uL RPMI)
6. Withdraw 10uL from the previous well into Eppendorf. Add in 10uL Trypan blue and vortex. Count cell. Record the cell number
7. Retreat the new wells with 1nm & 5nM PLK4i and incubate for another 3@4 days