

Side_Pop_Protocol

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Protocol

Note: Seed cells 1-2 days before so they are in log phase growth

Note: Preheat thermomixer to 37°C and take out Verapamil and Hoechst from -20°C before starting

1. Suspend cells and count
2. Dilute cells to 1×10^6 cells/mL
Note: Determine correct dilution media (FACS buffer, Media, ect.)
Ex: For N2 use media as FACS buffer will reduce viability
3. Distribute 1ml of cell suspension to eppendorfs
4. Add X μ L verapamil to control samples and X μ L PBS to test samples
Note: Verapamil volume depends on cell line; cell lines with larger morphology could require more volume. Important to optimise this aspect of the assay before proceeding.
Ex: 10 μ L Verapamil is optimal for N2 PDX
5. Incubate samples for 20 min at 37°C shaking at 600rpm
6. Add Hoechst to samples
Note: Be **VERY** precise with pipetting (ie. remove droplets from tip). This is the most important optimization step. Test with a range of concentrations with corresponding Verapamil controls to determine optimal volumes for both reagents.
Note: Protect Hoechst from light (ie. cover shaker with tinfoil, try to operate in the dark)
Ex: 2.5 μ L Hoescht is optimal for N2 PDX
7. Incubate samples in thermomixer for 2hrs at 37°C and 600rpm
8. Put samples on ice and spin down at 250 x g at 4°C
Note: Set centrifuge to 4°C 30min before the end of Hoechst incubation, collect ice and label FACS tubes
9. Resuspend in ice cold dilution media
Note: Use ~300 μ L
10. Read samples on Fortessa II

Reagents

Reagent	Product Code	[Stock]	[Assay]	Volume of Stock Used
Hoescht	ab228551	1mg/ml	2-5 $\mu\text{g/mL}$	2-5 $\mu\text{L/mL}$
Verapamil	V4629	10mM	100 μM	10 $\mu\text{L/mL}$

Cytometer Optics

- Email flow facilitates about changing the filters before starting
- UV Laser (Fortessa II) excites at 350nm
- Dichroic mirror LP600 splits beam:
 - Hoechst Blue: 450/50 BP
 - Hoechst Red: 610/20 BP