Side_Pop_Protocol

Callum Malcolm

2023-05-18

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 1x10^6 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 μL verapamil to control samples and X μL 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 μL 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 μL 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 μL
10. Read samples on Fortessa II

Reagents

Reagent	Product Code	[Stock]	[Assay]	Volume of Stock Used
Hoescht	ab228551	1mg/ml	2-5 μg/mL	2-5μL/mL
Verapamil	V4629	10mM	100μM	10μL/mL

Cytometer Optics

- Email flow facilites 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