70% Ethanol Fixation V.1

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This protocol is specifically written for fixing/permeabilizing suspension cell culture in a final volume of 1mL. For DNA content analysis, such as for cell cycle analysis, a precipitating fixative such as ethanol is preferred over formaldehyde fixation (Darzynkiewicz et al. 2001). The process of fixation preserves the cell state, makes cells permeable and allows for storage for extended periods of time.

Arnold Federico 2022. 70% Ethanol Fixation. **protocols.io**
https://protocols.io/view/70-ethanol-fixation-b724rqgw

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- 100% EtOH, pre-cooled in -20ºC
- 1.5mL microcentrifuge tubes
- 1X PBS
- Ice

Protocol

1 Transfer 1 * 10^6 to 5 * 10^6 suspended cells to appropriate centrifuge tube and pellet by spinning at 300g for 5 minutes

2 Discard supernatant without disturbing pellet. Resuspend and wash cells in 1mL 1X PBS with gentle pipetting. Spin down cells again at 300g for 5 minutes.

2.1 ▪ While cells are spinning, add 700uL of 100% EtOH (pre-cooled in -20ºC) to new 1.5mL microcentrifuge tube and keep on ice

3 Discard supernatant without disturbing pellet. Resuspend pellet in 300uL 1X PBS and gently pipette or vortex to break up cell clumps. (Cell aggregates will be stabilized when introduced to ethanol, make sure you have an even cell suspension!).

Transfer all 300uL cell suspension to microcentrifuge tube containing 700uL EtOH on ice and pipette mix to evenly distribute cells.

4 ▪ Transfer cells to 4ºC for at least 2hr prior to staining/analysis. Cells should be stable in -20ºC for months.