Cell Cycle Analysis
Protocol from Vindelov et al.
Cytometry 3, 323-327 (1983)

General Protocol for the isolation of Nuclei for Long Term Storage

1. Determine how many sub-confluent T-25 flasks to harvest. (General rule: One T-25 per group. For cell cycle analysis you will require 1 x 10^6 cells per treatment group).
2. Aspirate the media off the T-25 flask.
3. Wash each T-25 with 1.0ml trypsin and immediately remove the wash
4. Trypsinize the cells off the flask with 1.0ml fresh trypsin.
5. When cells are no longer adherent, inactivate the trypsin by adding 3.0ml fresh media.
6. Transfer similarly treated cells to a polypropylene tube and collect the cells by centrifugation for 5 minutes at 800 rpm in the tabletop centrifuge.
7. Carefully remove ALL of the media.
8. Add 200μl of citrate buffer and quickly resuspend the cell pellet. (Adjust to ≈ 5 x 10^6 cells/ml and transfer suspension to an Eppendorff tube).
9. Freeze cell suspension immediately in a dry ice EtOH bath.
10. When cells are frozen, store at -80°C for long term storage.

Preparation for staining

- Cut 30μM nylon mesh into small squares. Insert one piece of mesh into the barrel of a 3cc syringe. Attach a 18gauge needle. Prepare one syringe per treatment group to be analyzed.
- Quickly thaw nuclei and an appropriate number of cell cycle solutions A, B and C at 37°C. Place solutions A and B at room temperature. Keep solution C on ice protected from light.

General Staining method

1. Transfer 200μl of each nuclei suspension (5 x 10^5 cells) to a separate 2052 flow tube.
2. Add solution A to each tube and mix well. Incubate reaction for 10 minutes at room temperature with gentle mixing every few minutes.
3. Add solution B to each tube, mix well. Incubate reaction for 10 minutes at room temperature.
4. Add ice cold solution C to each tube. Mix samples and filter through nylon mesh into foil-wrapped flow tubes. Keep on ice.
5. Bring samples to flow facility 15 minutes to 3 hours after adding solution C.

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Preparation of Reagents

1. **Citrate Buffer:** 250mM sucrose, 40mM Citrate, 5% DMSO

Dissolve 85.50g sucrose and 11.76g trisodium citrate dihydrate in 800 ml dH_2O. Add 50ml DMSO. Adjust the volume to 1000ml with dH_2O. Adjust pH to 7.60

2. **Stock solution:** 3.4mM trisodium citrate dihydrate, 0.1% Nonidet P 40 (NP 40), 1.50 mM sperminetetrahydrochloride, 0.5mM Tris

Dissolve 2.0g trisodium citrate dihydrate, 2,0ml NP-40, 1.044 g spermine and 10121 g Tris in dH_2O to make a total volume of 2000 ml. Adjust pH to 7.60.

3. **Solution A:** 0.03mg/ml trypsin in stock solution

Dissolve 15mg trypsin (Sigma T-0134) in 500 ml stock solution. Adjust pH to 7.60.
For long term storage alliquot 10ml per screw cap tube and freeze at -80°C.

4. **Solution B:** 0.5mg/ml trypsin inhibitor, 0.1mg/ml ribonuclease A in stock solution.

Dissolve 250mg trypsin inhibitor (Sigma T-9253) and 50 mg Ribonuclease A (Sigma R-4875) in 500ml stock solution. Adjust pH to 7.60.

For long term storage, aliquot in 10ml per screw cap tube and freeze at -80°C

5. **Solution C:** 0.416 mg/ml propidium iodide, 1,16 mg/ml sperminetetrahydrochloride in stock solution.

KEEP SOLUTION PROTECTED FROM LIGHT DURING PREPARATION AND STORAGE

Dissolve 20.8 mg propidium iodide (Calbiochem) and 58mg sperminetetrahydrochloride in 50 ml stock solution. Adjust pH to 7.60

For long term storage, alliquot 10 ml per screw cap tube and freeze at -80°C.