

Metaphase Preparation

Optimized for Pro-B Lymphoma

I. Cell Incubation and Stimulation

1. Add 5×10^6 cells to the well of a 12-well plate containing 2mL of RPMI 1640 with IL-7 (25ng/mL).
2. Add colcemid (1:200), gently swirl plate, and incubate 2-3 hours.
3. Place 75mM KCl solution into water bath to pre-warm at 37°C.

II. Harvest and Swell Cells

1. Gently pipet cells out of plate and into 15mL conical tube with 2mL PBS at room temperature.
2. Invert tube and pellet cells at 1,000 rpm for 5 minutes.
3. Aspirate all but 100 μ L of supernatant.
4. Using a P200 pipet, gently resuspend the cell pellet.
5. Drop by drop, slowly begin to overlay 5mL of warm 75mM KCl with P1000 pipet, and then very gently pipet to mix solution.
6. Place tube in 37°C water bath, and incubate 20 minutes.
7. Prepare 3:1 methanol:acetic acid fix (40mL/sample) and place on ice.

III. Fix Cells

1. Remove tube from bath and add 5 drops cold fix to the sample.
2. Quickly invert, and pellet at 1,000 rpm for 10 minutes at room temperature.
Keep on ice as much as possible after this step.
3. Aspirate all but 100 μ L of supernatant, and flick pellet to resuspend.
4. Slowly (for the first few milliliters) add 10mL to the sample while vigorously rotating tube.
5. Invert tube and pellet at 1,200 rpm for 10 minutes at 4°C.
6. Aspirate all but 100 μ L of the supernatant, and flick to resuspend the pellet.
7. Repeat steps 4-6 two more times.
8. Aspirate all but 100-500 μ L and transfer to 1.5mL eppendorf tube.
9. Store metaphases at -20°C indefinitely.

Supplies and Reagents

- RPMI 1640
- IL-7 for Pro-B survival (R&D Systems 407-ML)
- 12-well tissue-culture treated plate
- Colcemid (Invitrogen15210-040)
- PBS
- 75mM KCl
- 3:1 methanol:acetic acid

Protocol: Metaphase Prep. For Pro-B Lymphoma
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