

## ***Preparation of metaphase chromosome spreads from adherent cells or lymphocytes.***

### *Equipment and reagents*

- Mouse Interleukin-2 (Boehringer Mannheim)
- Phytohemagglutinin M-form (Gibco BRL)
- *KaryoMax Colcemid* (Gibco BRL)
- Trypsin–EDTA (Gibco BRL)
- KCl solution (0.056 M or 0.075 M in H<sub>2</sub>O)
- Methanol:Glacial acetic acid at 3:1 freshly made each time
- 75 cm<sup>2</sup> tissue culture flasks
- Centrifuge conical tubes
- Microscopy slides
- Pasteur pipettes

### *Method for adherent cells*

1. Passage 10<sup>6</sup> cells/ml to a 75 cm<sup>2</sup> flask containing 5ml of regular growth medium 2 to 3 days prior to performing the chromosome spreads.
2. Feed cells with fresh media 12-14 h prior to harvesting.
3. Add Colcemid to a final concentration of 0.02 µg/ml; incubate at 37 °C for a 3-4 h.
4. Transfer the medium to a centrifuge tube and rinse the flask with 1.5 ml PBS (without Ca<sup>2+</sup> and Mg<sup>2+</sup>). Detach the cells with 0.5 ml of trypsin. Collect the cells and rinse the flask with 1.5 ml PBS 2 times.
5. Spin down the cells at 160 g for 10 min.
6. Aspirate the supernatant. Leave about 0.5 ml of supernatant and resuspend the pellet tapping the bottom of the tube gently with your finger.
7. Add 5 ml KCl 0.056 M solution. Pipette up and down to break any clumps. Leave at room temperature for 30 min.
8. Spin down the cells at 160 g for 10 min.

9. Aspirate the supernatant leaving 1 ml of the hypotonic solution. Resuspend pellet and add 1ml of methanol:glacial acetic acid (3:1) solution<sup>a</sup>. Spin down the cells at 160 g for 10 min.
10. Aspirate the supernatant and resuspend the pellet in 3 ml fixative again. Add slowly and mix the cells as before for the first 1ml.
11. Spin down the cells at 160 g for 10 min.
12. Aspirate the supernatant. Repeat steps 10-11 2 more times.
13. Resuspend pellet in small volume of fixative (typically less than 500µl), until the cell suspension looks slightly milky.
14. Take up a small quantity of cell suspension in the Pasteur pipette. Hold the pipette vertically with the end about 10 cm above the slide. Release a single drop from this height onto the slide. Let air dry<sup>b</sup>.

#### *Method for suspension cells*

1. Seed  $10^6$  cells/ml in a 75 cm<sup>2</sup> flask containing 5ml of regular growth medium supplemented with 100 µl of phytohemagglutinin and/or 20 U/ml of mouse interleukin-2<sup>c</sup>.
2. Incubate the cell culture at 37 °C for 72 h. Shake the flask gently 1-2 times per day.
3. Add Colcemid (10 µg/ml) about 45 min before harvesting.
4. Transfer the cells into a centrifuge tube.
5. Continue as in step 5 of *protocol for adherent cells*, but use KCl 0.075 M solution.

<sup>a</sup>It is very important to disrupt the pellet before addition of the fixative and to add the fixative only one or two drops at a time tapping the bottom of tube to mix the cells all the time.

<sup>b</sup>The room temperature and humidity are important factors to be considered when making the chromosome preparations. Chromosomes will spread poorly in a warm and dry environment. If necessary breath on the slide before dropping

the suspension. Alternatively, place the dry slides in the freezer for 30-60 min and drop the cell suspension onto these cold and wet slides.

°Phytohemagglutinin and mouse interleukin-2 are mitogenic agents. Human lymphocytes require only phytohemagglutinin, whereas mouse lymphocytes grow better with a combination of both of them.