Metaphase Preparation from Mouse Embryonic Fibroblast (MEF)

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Reagents

Acetic acid, glacial
Mallinckrodt, Cat. V193
Colcemid KaryoMAX Solution, 10 μg/ml
Invitrogen, Cat. 15210-016
DMEM
Invitrogen, Cat. 11960-044
Fetal Bovine Serum, heat inactivated
Invitrogen, Cat. 16140-022 (make 50 ml aliquots)
L-Glutamine-200 mM, 100X
Invitrogen, Cat. 25030-016 (make 5 ml aliquots)
Methyl alcohol, anhydrous
Mallinckrodt, Cat. 3016
Penicillin/Streptomycin
5,000 U/ml/5,000 μg/ml
Invitrogen, Cat. 15070-014 (make 5 ml aliquots)
or
Antibiotic-Antimycotic
10,000 U/ml Penicillin G sodium; 10,000 μg/ml streptomycin sulfate; 25 μg amphotericin B,
Invitrogen, Cat. 15240-013 (make 5 ml aliquots)
Phosphate buffered saline (PBS), 1X
Potassium chloride (KCl)
Mallinckrodt, Cat. 6858
Trypsin-EDTA
Invitrogen, Cat. 25200-056

Preparation

Complete Media (final concentrations)

DMEM	
L-Glutamine	f.c. [2 mM]
Penicillin	f.c. [50 U/ml]
Streptomycin	f.c. [50 μg/ml]
FBS	f.c. [10%]

Hypotonic Solution
0.56 g KCl /100 ml distilled water

Fixative
Methyl alcohol/Acetic acid, 3:1(volume to volume)
Prepare fresh prior to use.

Procedure

DAY 1

1. Culture the cells in complete media in a 6 or 10 cm petri dish.

2. For best results, split cells lightly (i.e., 1:2 or 1:4) so that the cells will be happily growing the following day; incubate cells at 37˚C overnight.

DAY 2

3. Add 10 µl Colcemid per ml media [f.c. 0.1 µg/ml], incubate at 37˚C for 3-5 hr.

4. Wash cells with 1X PBS to remove media and serum.

5. Remove cells with trypsin.

6. Pellet at 1200 rpm for 8 min in 15 ml conical tube.

7. Remove supernatant, leaving 0.5 ml.

8. Resuspend cells thoroughly by pipeting.

9. Add 10 ml prewarmed 0.075M KCl (37˚C) (hypotonic solution)
   Note: The key to this step is to slowly (drop by drop) add the KCl, otherwise cell clumps will form which are impossible to disperse. For example, add a few drops and then pipet the cells up and down to thoroughly mix them, and then add a few more drops, etc. (as the volume increases in the tube, you can add more between mixes).

10. Incubate at 37°C for 15 min, inverting tube a few times during the incubation.

11. Add a few drops (literally) of fresh fixative (methanol:glacial acetic acid, 3:1).

12. Pellet cells at 1200 rpm for 8 min.

13. Remove supernatant, leaving 0.5 ml.

14. Resuspend cells completely by pipeting (BE GENTLE – CELLS ARE LIKE

WATER BALLOONS AND VERY FRAGILE).

15. Slowly add fresh fixative solution in the same manner as the KCl solution in step 9.

16. Pellet cells at 1200 rpm for 8 min.

17. Remove supernatant, leaving 0.5 ml and resuspend cells thoroughly by pipeting.

18. Repeat steps 11-13 (fixative need not be added as slowly after first fixation) until cells are in their third fixation.

19. Cells can be stored at 4˚C, shipped on ice, or metaphases directly prepared.