Metaphase Preparation from Mouse/ Rat Spleen

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Acetic acid, glacial
Mallinckrodt, Cat. V193
Antibiotic-Antimycotic, 100X
10,000 U/ml Penicillin G sodium; 10,000 μg/ml streptomycin sulfate;
25 μg amphotericin B,
Invitrogen, Cat 15240-013
or
Penicillin/ Streptomycin
5,000 U/ml/5,000 μg/ml
Invitrogen, Cat.15070-014
β-Mercaptoethanol
Sigma, Cat. 6250
Concanavalin A, 5 mg
Sigma-Aldrich, Cat. C-5275
Colcemid, KaryoMAX Colcemid Solution, 10 μg/ml
Invitrogen, Cat. 15210-016
Fetal Bovine Serum (FBS) Qualified, heat inactivated
Invitrogen, Cat. 16140-022
Homogenizer
Thomas Scientific, Cat. 3431D7
L-Glutamine-200 mM, 100X
Invitrogen, Cat. 25030-016
Lipopolysaccharides (LPS), 5 mg
Sigma-Aldrich, Cat. L-2637
Methyl alcohol, anhydrous
Mallinckrodt, Cat. 3016
Potassium Chloride (KCl)
Mallinckrodt, Cat. 6858
RPMI Medium 1640
Invitrogen, Cat. 21870-050
Water, sterile tissue culture grade

Preparation

Concanavalin A (5mg)
Stock solution: 5 µg/µl in tissue culture grade water
Store aliquots at –20°C

Lipopolysaccharides (LPS) (5mg)
Stock solution: dissolve 5 mg in 1 ml sterile water
Use 2.5:997.5 dilution for a final concentration of 25 µg/ml of culture

0.5% Beta-Mercaptoethanol
2µl 100% B-M, 400 µl sterile water

Hypotonic Solution (0.075M KCl)

KCl	5.6 g
Distilled water	1000 ml

Pre-warm to 37˚C

Fixative
Prepare fresh methanol:acetic acid, 3:1, volume:volume

Medium, complete

RPMI 1640	500 ml
Antibiotic –Antimycotic 100X	5 ml
or	
Penicillin/Streptomycin	5 ml
L- glutamine	5 ml

Do not add FBS!!
Filter sterilize solution

Procedure

1. Transport spleen in medium w/o FBS.

2. Place a single spleen into a homogenizer with 3 ml of prepared medium w/o FBS. Grind well. Add 7 ml of complete medium to homogenizer.

3. Centrifuge at 1000 rpm for 10 min.

4. Remove supernatant and add 4 ml of complete medium to the tube.

5. Transfer 0.5 ml to one T75 flask.

6. Add 20 ml of complete RPMI 1640 media.

7. Add the following to this flask:

FBS	2.5 ml
Concanavalin A, 5 µg/ul	30 µl

Lipopolysaccharides (diluted)	25 µl
βMercaptoethanol, 0.5%	30 µl

8. Incubate flask at 37°C for 48 hr. Check under the microscope: cells should be growing in clumps; if there are no clumps, incubate an additional 24 hr.

9. At the end of the 48 hr incubation, add 0.25 ml Colcemid to each flask. Continue to incubate for an additional 30 min until cells start to divide.

10. Transfer the suspension to 50 ml tubes.

11. Centrifuge at 1000 rpm for 10 min.

12. Remove supernatant leaving 500 µl of solution.

13. Resuspend pellet.

14. Gently add, drop-wise, 5 ml 0.075 M KCl, prewarmed to 37°C.

15. Incubate at 37°C for 15 min.

16. Add a few drops of freshly prepared fixative.

17. Centrifuge at 1000 rpm for 10 min.

18. Remove supernatant to 500 µl.

19. Resuspend pellet.

20. Transfer to 15 ml tubes.

21. Add fixative to 10 ml.

22. Centrifuge at 1200 rpm. Repeat wash with fresh fixative at least 3 times.

23. Remove the supernatant one final time and drop 12 µl of the suspension onto a clean slide in the humidity of a drying chamber or Thermotron.

24. The slides are stored in a slide box in a drawer at room temperature for one week, then sealed in a heat-sealable pouch (e.g. Kapak/Scotchpak) with Drierite until use.