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Metaphase Preparation from Mouse/ Rat Spleen

Section of Cancer Genomics, Genetics Branch, NCI
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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 Gibco BRL, Cat 15240-013
or
Penicillin/ Streptomycin
5,00 U/ml/5,000 μg/ml
Gibco BRL, Cat.15070-014
β-Mercaptoethanol
Sigma, Cat. 6250
Concanavalin A, 5 mg
Sigma-Aldrich, Cat. C-5275
Colcemid, KaryoMAX Colcemid Solution, 10 μg/ml
Gibco BRL, Cat. 15210-016
Fetal Bovine Serum Qualified, heat inactivated
Gibco BRL, Cat. 16140-022
Homogenizer
Thomas Scientific, Cat. 3431D7
L-Glutamine-200 mM, 100X
Gibco BRL, Cat. 25030-016
Lipopolysaccharides (LPS), 25 mg
Sigma-Aldrich, Cat. L-2637
Methanol alcohol, Anhydrous
Mallinckrodt, Cat. 3016
Potassium Chloride (KCl)
Mallinckrodt, Cat. 6858
RPMI Medium 1640
Gibco BRL, Cat. 21870-050

Preparation

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

Lipopolysaccharides (LPS) (25mg)
Stock solution: dissolve 25 mg in 1 ml sterile water
Use 1:1000 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	5.6 g
Distilled water	100 ml

Pre-warm to 37˚C

Fixative
Prepare fresh methanol:acetic acid, 3:1

Medium

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.

3. Transfer 0.5 ml to one T75 Nunc flask.

4. Add 20 ml of prepared media.

5. Add the following to this flask:

FBS	2.5 ml
Concanavalin A, 5 µg/ul	30 µl
Lipopolysaccharides (diluted)	25 µl
β-Mercaptoethanol, 0.5%	30 µl

6. Incubate flask at 37°C for 48 hours.

7. At the end of the 48-hour incubation, add 0.25 ml Colcemid to each flask. Continue to incubate for an additional 30-60 min until cells start to divide.

8. Transfer the suspension to 50 ml tubes.

9. Centrifuge at 1,000 rpm for 10 min.

10. Remove supernatant leaving 500 µl of solution.

11. Resuspend pellet.

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

13. Incubate at 37°C for 20 minutes.

14. Add 1 ml of freshly prepared fixative.

15. Centrifuge at 1,000 rpm for 10 minutes.

16. Remove supernatant to 500 µl.

17. Resuspend pellet.

18. Transfer to 15 ml tubes.

19. Add fixative to 10 ml.

20. Centrifuge at 1,000 rpms. Repeat wash with fresh fixative at least 3 x.

21. 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.

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