Appendix

The solutions

1. **β-Glycerophosphate 1 M, 50 ml**

β-Glycerophosphate	10.8 g
Sterile distilled water	50 ml
Sterile filter	

2. **β-Glycerophosphate 10 mM in culture medium**

β-Glycerophosphate 1 M	100 µl
Culture medium.	10 ml
Aliquot 1000 µl at -20°C	

3. **1α,25-Dihydroxychloecalciferol (VD3) 100 nM stock solution, 240 µl**

VD3	10 µg
Absolute ethanol	240 µl

4. **1α,25-Dihydroxychloecalciferol (VD3) 10nM in culture medium**

VD3 100 nM	1 µl
Culture medium	10 ml

5. **2-Amino-2-methyl-1-propanol 0.75 mM, 1 L**

2-Amino-2-methyl-1-propanol	7.19 g
Deionized water	1 L
Adjust pH to 10.3 using 1M NaOH	

6. **Acetic acid 2.5N /10M urea, 250ml**

Urea	150.2 g
Deionized water	100 ml.
Glacial acetic acid	35.9 ml
Adjust final volume to 250 ml	

7. **Alcian blue solution, 100 ml**

Alcian blue	1.0 g
Glacial acetic acid 3%	100 ml

8. **Antibody diluting solution (1% Bovine serum albumin and 0.1% Sodium nitrite), 10 ml**

Bovine albumin	1 g
Sodium nitrite (NaN_3)	0.1 g
DPBS	10 ml

9. **Ascorbic acid 5 mg/ml, 50 ml**

Ascorbic acid	0.25 g
Deionized water	50 ml
Sterile filtratation	
Aliquot 1000 µl at -20°C	

10. Ascorbic 50 μg/ml in culture medium

Ascorbic acid 5 mg/ml	100 μl
Cuture medium	10 ml of culture medium.

11. Bovine thrombin 5000 Unit/ml stock solution, 2 ml

Bovine thrombin	1000 Unit
Distilled water	2 ml
Aliquot 200 μl and store at -20°C	

12. $CaCl_2$ 20% stock solution, 100 ml

$CaCl_2$	20 g
Distilled water.	100 ml
Sterile filered using 0.2 μm pore filter	

13. Chloroform/Methanol, 3:1, Solution, 4 L

Chloroform	3 L
Methanol	1L
Mix well under chemical hood	

14. Dexamethasone 500μM (200 ng/μl) stock solution, 5 ml

Dexamethasone	1 mg
Absolute ethanol	5 ml

15. Dexamethasone 20 nM in culture medium

Dexamethasone 500 μM	2 μl
Culture medium	50 ml

16. EDTA 0.5M pH 8.0, 50 ml

NaOH 10 N	50 ml
EDTA	186.12 g (0.5 mol)

Under hood with continuous stirring, Adjust pH to 8.0 using 30% HCL

17. Electrophoresis gel loading buffer, 10 ml

Glycerin	3.73 ml (50% v/v)
Xylencyanol	25.0 mg (0.25% w/v)
Bromphenolblau	25.0 mg (0.25% w/v)
Distilled water	6.27 ml

Sterile filtered

Store at -20°C for long term storage and at 4 °C for current usage

18. Electrophoresis 0.2% gel, 300 ml

Agarose	6 g
Tris-acetic-EDTA buffer (TAE) x 1	300 ml

Boiling in microwave at high level

19. Ethidiumbromide 0.001%, 200 ml

Ethidiumbromide	1 %
Deionized water	200 ml

20. GuHCL 4M /50mM Tris-HCL-solution, 4 L

GuHCL	1528.48 g
Tris	24.24 g
Deionized water	3.5 L

Continuous stirring until dissolve

Adjust volume to 4.0 L and Adjust pH to 7.4 with 25% HCL.

21. Lysis buffer in ALP activity analysis

2-Amino-2-methyl-1-propanol 0.75 mM	1ml
p-Nitrophenylphosphate (add Just before use add)	2 mg
Vortex briefly for mixing	

22. Masson-Trichrom-Goldner staining

Weigerts Eisenhaematoxylin

Weigerts solution A and solution B	1:1

Masson solution (Goldner I)

Solution A	33 ml
Solution B	66 ml
Solution C	20 ml, mix well
Acetic acid 0.2%	880 ml

Solution A:

Acidfuchsin	1 g
Acetic acid	1 ml
Deionized water	100 ml

Solution B:

Ponceau Xylidin	1 g
Acetic acid 100%	1 ml
Deionized water	100 ml

Solution C:

Azophloxin	0.5 g
Acetic acid 100%	0.2 ml
Deionized water	100 ml

Orange-G solution

Orange G	10 g
Deionized water	500 ml
Continuous stirring until dissolve, then add	
Molybdatophosphoric acid	15 g

Light green

Light green	0.5 g
Acetic acid 100%	1 ml
Deionized water	500 ml

23. Mineralized culture medium

DMEM-F12	500 ml
Fetal bovine serum (FBS) (10%)	50 ml
Pennicillin / Streptomycin solution (1%)	1 ml
Fungizone (0.1%)	0.5 ml
β-Glycerophosphate	10 mM
Ascorbic acid	50 μg/ml

24. NaOH 10N, 100 ml

NaOH pellet	4 g
Deionized water	100 ml

Continuous stirring using magnetic bar in plastic container

25. NaOH 2.7 N /1M HEPES, 250 ml

NaOH 10 N	67.5 ml
HEPES	59.5 g
Deionized water	140 ml

Continuous stirring and Adjust volume to 250 ml using deionized water

26. Neutral Buffered Formalin 10%, 1 L

Sodiumphosphate, monobasic	4 g
Sodiumphosphate, dibasic	6.5 g
Formaldehyde 37%	100 ml
Continuous stirring	
Adjust pH to 6.8-7.0 with 10M NaOH	

27. Neutral red solution 0.005% in culture medium

Neutral red solution 0.5%	100 µl (sterile filtered)
Culture medium	10 ml
Mix well	

28. Nuclear Fast Red

Aluminium sulfate hydrated	25.0 g
Nuclear fast red	0.5 g
Deionized water	500 ml
Continuous stirring using magnetic bar	

29. Oil Red O solution,

Oil Red O	0.7 g
Propylene glycol	100 ml
Continuous stirring at 70°C	

30. p-Nitrophenol solution 10 µM – 250 µM (Standard solution for ALP activity measurement) 1 L

p-Nitrophenol	10-250 µmol
Buffer for standard solution	1 L
Glycin	100 mM (7.507 g/L)
$ZnCl_2$	1mM (0.13639 g/L)
$MgCl_2$	1 mM (0.2033 g/L)
Deionized water	
Adjust pH to 9.6	
P-Nitrophenol solution 10 µM – 250 µM	

31. Poly D-Lysin 1 mg/ml stock solution, 10 ml

Poly-D-Lysin	10 mg
DPBS	10 ml
Store at -20°C	

32. Propylene glycol 85%, 100 ml

Propylene glycol	85 ml
Deionized water	15 ml

33. Silver nitrate solution 5%, 100 ml

Silver nitrate	5 g
Distilled water	100 ml

34. Sodiumthiosulfate 5%

Natriumthiosulfate	5 g
Deionised water	100 ml

35. Thrombin 1000 Unit in 10% $CaCl_2$ solution, 100 µl

Bovine thrombin 5000 Unit/ml	10 µl
$CaCl_2$ 20%	50 µl
Distilled water	40 µl

36. Toluidine blue staining solutions (Solution A and Solution B)

Solution A:

Di-soodium tetraborate	8 g
Toluidine blue	8 g
Deionized water	800 ml

Stirring for 15 minutes

Solution B:

Pyronin G	2 g
Deionized water	200 ml

Stirring for 15 minutes

37. Tris-Acetic acid-EDTA (TAE) x50 buffer, 500 ml

Tris	121 g
Acetic acid 100%	28.55 ml
EDTA 0.5M	50 ml

Continuous stirring for complete dissolving

Adjust volume to 500 ml using deionized water

38. Tris buffer saline (0.5 M), x10, 1 L

NaCl	87.8 g
Tris	60.6 g
Deionized water	1 L

Adjust pH to 7.5 using 10% HCL.

39. Triton in PBS 0.2% solution, 1 ml

Triton X-100	2 µl
DPBS	1000 µl

VITAE

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