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-Dihydroxycholecalciferol (VD3) 100 nM stock solution, 240 µl
   - VD3: 10 µg
   - Absolute ethanol: 240 µl

4. 1α,25-Dihydroxycholecalciferol (VD3) 10 nM 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, 250 ml

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 (NaN₃) 0.1 g
DPBS 10 ml

9. Ascorbic acid 5 mg/ml, 50 ml

Ascorbic acid 0.25 g
Deionized water 50 ml
Sterile filtration
Aliquot 1000 µl at -20°C
10. Ascorbic 50 μg/ml in culture medium

Ascorbic acid 5 mg/ml 100 μl
Culture 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. CaCl₂ 20% stock solution, 100 ml

CaCl₂ 20 g
Distilled water 100 ml
Sterile filtered using 0.2 μm pore filter

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

Chloroform 3 L
Methanol 1 L
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)
Xylen cyanol 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

Weigert's Eisenhaematoxylin
Weigert's 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 Xyldin 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
Penicillin / 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

Sodium phosphate, monobasic 4 g
Sodium phosphate, 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)
ZnCl₂ 1 mM (0.13639 g/L)
MgCl₂ 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% CaCl₂ solution, 100 µl

- Bovine thrombin 5000 Unit/ml 10 µl
- CaCl₂ 20% 50 µl
- Distilled water 40 µl

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

**Solution A:**
- Di-sodium 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

DPBS

2 μl

1000 μl
VITAE

Name
Miss Premjit Arpommaeklong

Birth date
22 June 1965 (Samuth Songkarm)

Educational Attainment

<table>
<thead>
<tr>
<th>Degree</th>
<th>Name of Institution</th>
<th>Year of Graduation</th>
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<td>Doctor of Dental Surgery (DDS)</td>
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<td>1989</td>
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<tr>
<td>Master of Dental Science (MDSc)</td>
<td>The University of Melbourne, Australia</td>
<td>1996</td>
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<tr>
<td>National Board Dental Examinations</td>
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<td>1997</td>
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Scholarship Awards during Enrolment

German Academic Exchange Service (DAAD) (Codenumber: A/99/16805)
Date
March 1999 – October 2001

Work-Position and Address:

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<tr>
<td>Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Prince of Songkla University, SONGKHLA</td>
<td>1989</td>
<td>Lecturer</td>
</tr>
<tr>
<td></td>
<td>1999 - date</td>
<td>Assistant Professor</td>
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