

## VIII. APPENDIX

### I. Reagents for Analysis of Alkaline phosphatase activity

(1.) 1 mol/L HCl

HCl (12.01 N) 83.2 mL.

- Filled up with distilled water to 1,000 mL.
- Stored at room temperature.

(2.) 0.84 mol/L 2-Amino-2-methyl-1-propanol Buffer  
(AMP buffer) pH 10.3

- Dissolved 75 g of AMP ( $C_4H_{11}NO$ ; MW 89.14) in  $CO_2$ -free water to 500 mL.
- Added 150 mL of 1 mol/L HCl, mixed well.
- Adjusted pH to 10.3 with 1 N HCl or 1 N NaOH.
- Filled up with  $CO_2$ -free water to 1,000 mL.
- Stored at 4° C in the dark.

(3.) 150 mmol/L stock  $MgCl_2$  solution

- Dissolved 3 g of  $MgCl_2 \cdot 6H_2O$  (MW 203.31) in  $CO_2$ -free water to make 100 mL of solution, mixed well.
- Stored at room temperature.

(4.) 1.5 mmol/L fresh working solution of  $MgCl_2$

- Diluted 1.0 mL of 150 mmol/L stock  $MgCl_2$  solution in  $CO_2$ -free water to make 100 mL of solution, mixed well.
- The solution will keep with negligible decomposition for about 7 d at 4° C.

(5.) 215 mmol/L PNPP substrate solution

- Dissolved 4 g of 4-Nitrophenyl phosphate (MW 371.15) in 1.5 mmol/L  $MgCl_2$  to make 50 mL of solution, mixed well.
- Aliquoted to vials (5 mL/vial)
- Stored at -20° C.

## **II. Reagents for determinations of physicochemical properties of the ALP isoenzymes**

### **A. Reagents for chemical inhibition of the ALP isoenzymes**

(1.) 500 mmol/L L- Phenylalanine

- Dissolved 8.26 g of L- Phenylalanine (MW 165.2) in distilled water to make 100 mL of solution, mixed well.
- Stored at 4° C.

(2.) 5 mmol/L Levamisole

- Dissolved 0.1204 g of Levamisole (MW 240.8) in distilled water to make 100 mL of solution, mixed well.
- Stored at 4° C.

### **B. Reagents for WGA precipitation of bone ALP isoenzyme**

(1.) 50 mM acetate buffer pH 4.5

CaCl <sub>2</sub>	0.8880	g
NaCl	7.8975	g

- Added distilled water to about 900 mL and mixed until well dissolved.
- Added 3 mL of glacial acetic acid, mixed well.
- Adjusted pH to 4.5 with 1 N HCl or 1 N NaOH.
- Filled up with distilled water to 1,000 mL.
- Stored at 4° C.

### **C. Reagents for screening of ALP isoenzymes by cellulose acetate electrophoresis**

(1.) Barbital buffer , pH 8.8

Sodium barbital	8.857	g
Barbituric acid	0.9735	g
Calcium lactate	0.383	g

- Added distilled water to about 900 mL and mixed until well dissolved.
- Adjusted pH to 8.8 with 1 N NaOH or 1 N Calcium lactate.

- Filled up with distilled water to 1,000 mL.
- Stored at 4° C.

(2.) Alkaline phosphatase indolyl blue reagent (kit)

- Reconstituted each vial of reagent with 3 mL of diluent, mixed well.
- The reagent may be used as soon as reconstituted or within 48 h.

(3.) 5% (v/v) acetic acid

**III. Reagents for fractionation of ALP isoenzymes by anion exchange chromatography**

(1.) 5 mmol/L Tris -HCl

Tris-HCl (MW 157.59)                      0.7879                      g

- Added distilled water to about 950 mL.
- Checked pH and stored at room temperature.

(2.) 100 mmol/L NaCl in 5 mmol/L Tris -HCl, pH 8.0

NaCl (MW 58.5)                              5.85                              g

- Added 5 mmol/L Tris -HCl to about 900 mL, mixed well.
- Adjusted pH to 8.0 with 1 N HCl.
- Filled up with distilled water to 1,000 mL, stored at 4° C.

(3.) 150 mmol/L NaCl in 5 mmol/L Tris -HCl, pH 8.0

NaCl (MW 58.5)                              8.775                              g

- Added 5 mmol/L Tris -HCl to about 900 mL, mixed well.
- Adjusted pH to 8.0 with 1 N HCl.
- Filled up with distilled water to 1,000 mL, stored at 4° C.

(4.) 300 mmol/L NaCl in 5 mmol/L Tris -HCl, pH 8.0

NaCl (MW 58.5)                              17.55                              g

- Added 5 mmol/L Tris -HCl to about 900 mL, mixed well.
- Adjusted pH to 8.0 with 1 N HCl.
- Filled up with distilled water to 1,000 mL, stored at 4° C.

#### IV. Reagents for electrophoresis on agarose gel

(1.) Tris-barbital-sodium barbital buffer (kit), pH 8.8-9.0

- Dissolved one package in 1,000 mL distilled water.
- Stored at 4° C.

(2.) 0.5% Agar for coated plastic plate

Agar	0.125	g
Distilled water	25	mL

- Boiled agar, and then left in water bath at 56° C before pour it on a plastic plate about 2 mL/plate.
- Dried the gel at 37° C until the surface was dry.

(3.) 0.8% Agarose

Agarose	0.2	g
Tris-barbital-sodium barbital buffer	25	mL

- Boiled agarose, and then left in water bath at 56° C before pour it on the precoted plastic plate (2) about 7 mL/plate.
- Left to stand at room temperature approximately 30 min.

#### V. Reagents for preparation of asialo-ALP isoenzymes

(1.) 1 unit/mL Neuraminidase (E.C.3.2.1.18) *Clostridium perfringens* (C-Neu)

- Diluted (v/v) 2 unit/mL of stock C-Neu with distilled water at a ratio 1: 2.

(2.) 125 mU/L Neuraminidase (C-Neu)

- Diluted (v/v) 2 unit/mL of stock C-Neu with distilled water at a ratio 1: 16.

(3.) 125 mU/L Neuraminidase from *Vibrio cholerae* (V-Neu)

- Diluted (v/v) 2 unit/mL of stock V-Neu with distilled water at a ratio 1: 16.

## **VI. Reagents for measurement of total sialic acid (TSA)**

- (1.) 1.3 mM periodic acid
  - Dissolved 0.0296 g of periodic acid (MW 227.94) in distilled water to make 100 mL of solution, mixed well.
  - Stored at 4° C.
  
- (2.) Stock 6 g/dL resorcinol reagent
 

Resorcinol	1.5	g
Stock 25 mg % CuSO <sub>4</sub>	1	mL
28 % HCl	15	mL
Distilled water	9	mL

  - Mixed all ingredients together until well dissolved.
  - Stored at -20° C.
  - Warmed the solution at room temperature before used.
  
- (3.) 0.6 g/dL working resorcinol reagent
  - Diluted (v/v) stock 6 g/dL resorcinol reagent with distilled water at a ratio 1: 10.
  
- (4.) Stock 25 mg % CuSO<sub>4</sub>
  - Dissolved 0.025 g of CuSO<sub>4</sub> in distilled water to make 100 mL of solution.
  - Mixed well and stored at room temperature.

## **VII. Reagents for sialylation of asialo-ALP isoenzyme**

- (1.) Sodium phosphate buffer 0.5 M, pH 6.8
  - Dissolved 7.8005 g of sodium dihydrogen phosphate (MW 156.01) in distilled water to about 80 mL.
  - Adjusted pH to exactly 6.8 with 1 N NaOH.
  - Filled up with distilled water to 100 mL, stored at 4° C.
  
- (2.) 2% (v/v) Bovine serum albumin (BSA)
  - Dissolved 0.2 g of BSA in distilled water to 10 mL.
  - Pretreated at 52° C for 40 min.
  - Stored at 4° C.

- (3.) 60 mmol/L Cytidine-5'-monophosphate - N-acetylneuraminic acid (CMP-NeuNAc)
- Mixed 18  $\mu$ L of 162.73 mmol/L stock CMP-NeuNAc with 32  $\mu$ L of sodium phosphate buffer 0.5 M, pH 6.8
  - Stored at -20° C.
- (4.) 1.5 units/L Sialyltransferase (ST)
- Mixed 10  $\mu$ L of 15 units/L stock sialyltransferase with 90  $\mu$ L of distilled water.
  - Stored at -20° C.

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