APPENDIX-VI

Composition of buffers-media used in ELISA, Western Blotting and antiechinococcal assessment:

Composition of PBS (pH-7.2)

Disodium Hydrogen Phosphate- 1.09gm
Potassium Dihydrogen orthophosphate- 0.2gm
Nacl- 8gm
Potassium chloride- 0.1gm
Distilled water- 1000ml

Composition of PBST (pH-7.2)

Add 500µl of Tween-20 in the above mentioned PBS recipe.

Composition of Antigen Coating buffer:

Carbonate bicarbonate buffer (CO$_3$HCO$_3$)
Sodium Hydrogen Carbonate (NaHCO$_3$)- 1.428 gm
Sodium Carbonate (Na$_2$CO$_3$)- 0.848 gm
Distilled water- 1litre
pH- 9.6

Composition of Washing Buffer:

Conc. Form of solution A:
Disodium Hydrogen orthophosphate (Na$_2$HPO$_4$)- 21.3 gm
(0.15 molar solution)
Distilled water – 1 liter

Conc. Form of solution B:
Potassium Dihydrogen orthophosphate (KH$_2$PO$_4$)- 20.4 gm
(0.15 molar solution)
Distilled water- 1 liter

Composition of Citric Phosphate Buffer (pH-4.5)

Disodium Hydrogen Phosphate- 6.2gm
Citric Acid- 5.1gm
Distilled water- 1000ml
**Blocking solution: 2% BSA**

BSA powder- 600 mg  
PBS with Tween-20

**Substrate Buffer: OPD- Citric Phosphate Buffer:**  
Disodium Hydrogen orthophosphate (Na$_2$HPO$_4$)- 6.2 gm  
Citric acid- 5.1 gm  
Distilled water- 1 Litre  
pH- 4.5

A) **2% Sodium Carbonate (Na$_2$CO$_3$) in 0.1N NaOH**  
Na$_2$CO$_3$ – 1 gm  
NaOH – 0.2 gm  
Distilled water – 50 ml

B) **CuSO$_4$- 5mg** (dissolved in 1 ml of distilled water before dilution)  
Potassium Sodium tartarate – 10mg

**Working Solution:**  
Solution A - 50 ml  
Solution B – 0.5 ml

C) **BSA Standard- 1mg/ml**  
D) **Folin & Ciocalteus regents (2N)** dilute 1 in 2 in d/w  

**Anti-Human IgG, (Whole Molecule)- Peroxidase Antibody produced in goat**  
A 8667 Sigma-Aldrich

Anti-Human IgG1, HRP conjugate antibody produced in goat  
1094391 Invitrogen

Anti-Human IgG2, HRP conjugate antibody produced in goat  
1118846A Invitrogen

Anti-Human IgG3, HRP conjugate antibody produced in goat  
1209861A Invitrogen
Anti-Human IgG3, HRP conjugate antibody produced in goat
929522 Invitrogen
1Mol NaOH
40 gm of NaOH pallets dissolved in 1000 ml of d/w.

70% Ethanol
70ml ethanol dissolved in 30 ml d/w.

96% Ethanol
96 ml ethanol dissolved in 4 ml d/w.

Primers: (Eurofins Genomics India Pvt Ltd)

a) NADH dehydrogenase I (nd-1):
   Forward: TGGTTTGTTGCAGAGGTTTG
   Reverse: TGGCGTACGATTAGTTTCACA

b) Cytochrome oxidase subunit I (cox I):
   Forward: GCTGGTGTTGGTTGGACATT
   Reverse: ACCAAATCCAGGCAAAATCA

Composition of Reagents used in Western Blotting

12.5% Separating gel

30% Acrylamide, 0.8% Bisacrylamide- 4.2 ml
d/w- 3.3 ml
1.5 M Tris (pH 8.8), 0.4% SDS- 2.5 ml
TEMED- 5 µl
10% APS- 50 µl

4% Stacking gel

30% Acrylamide, 0.8% Bisacrylamide- 1.3 ml
d/w- 6.1 ml
1.5 M Tris (pH 8.8),
10% SDS- 100µl
TEMED- 10 µl
10% APS- 50 µl
**Dulbecco's Modified Eagle's Medium**

10% FCS  
Antibiotics (100 IU/mL of penicillin and 50μg/mL of streptomycin)  

**RPMI 1640**

12mM HEPES  
2mM glutamine  
200 U/mL of penicillin  
200mg/mL of streptomycin  
0.50mg/mL amphotericin B  
10% FCS  
4mg/ml glucose