Appendix

1. 0.2 M phosphate buffered saline (PBS) stock, pH 7.4

Solution A
NaH$_2$PO$_4$.2H$_2$O (Qualigens fine chemicals, U.S.A) 3.12 gm
Double distilled water (DDW) 100 ml

Solution B
Na$_2$HPO$_4$ (Qualigens fine chemicals, U.S.A) 2.84 gm
DDW 100 ml

Mix 19 ml of solution A and 81 ml of solution B. Make up the volume to 200 ml with DDW. Adjust the pH to 7.4. Then add 0.9% NaCl to the solution and adjust the pH. The solution was autoclaved at 15 lbs per square inch (psi) for 25 min and stored at 4ºC.

Working PBS (0.01M): 25 ml 0.2M PBS + 475 ml DW

2. Carbonate bicarbonate buffer

A] 0.2 M solution of anhydrous sodium carbonate (Na$_2$CO$_3$) M.W 105.99
2.12 gm in 100 ml DW

B] 0.2 M solution of sodium bicarbonate (NaHCO$_3$) M.W 84.01
1.68 gm in 100 ml DW

Stock buffer: 16.9 ml of A + 37 ml of B = 53.9 ml (pH 9.5)

Working buffer: Dilute the stock buffer solution 1:4 (1 ml stock buffer + 3 ml DW)

3. Wash buffer (PBST)

Add 0.05% Tween 20 to 0.01 M PBS pH 7.4

4. Post coating reagent in ELISA tests

1.2% BSA: 1.2 gm of BSA dissolved in 100 ml of PBS-T
5% SMP: 5 gm of SMP dissolved in 100 ml of PBS-T

5. Diluent in ELISA tests

2.5% SMP: 2.5 gm of SMP dissolved in 100 ml of PBS-T

6. Stop solution for ELISA tests (1 M H$_3$PO$_4$)

Phosphoric acid 28 ml
DDW 472 ml
7. **Penicillin-Streptomycin**

Penicillin (500,000 Units each vial)  2 vials
Streptomycin  1.00 gm
DW  10.00 ml

Inject 2.5 ml DW in penicillin vial and 5 ml in streptomycin vial. Pool both the antibiotics and make up the volume to 10 ml. Make aliquots of 1 ml and store at –20°C.

8. **Earle’s MEM**

MEM [E] 1 vial + DW 928.0 ml, stir well and divide the solution equally into two bottles. To this solution add 2.5 ml of Ferric Nitrate (Fe(NO₃)₃·9H₂O (0.002%)). Autoclave at 1 lbs/10 min/115ºC. Add 28.60 ml of sterile sodium bicarbonate (NaHCO₃) (3.5%), adjust the pH to 7.2-7.4. Test the medium for sterility. Store at 4C. Mix 5 ml glutamine (3%) and 1 ml penicillin (200 U/ml) and streptomycin (200 gm/ml) to 500 ml of medium just before use.

9. **TPCK treated trypsin (1mg/ml)**

Dissolve 20 mg of TPCK treated trypsin powder + 20 ml of DW. Make aliquots of 100 µl and 500 µl and store at -20ºC.

10. **Solutions for Lowry’s method of protein estimation**

(i) **Standard BSA at 1 mg/ml**
10 mg of BSA was dissolved in 10 ml of DW

(ii) **Solution A**
10 gm Na₂CO₃ (BDH) was dissolved in 500 ml 0.1 NaOH

(iii) **Solution B**
0.5 gm CuSO₄ was dissolved in 1% sodium citrate

(iv) **Solution C**
50 ml of solution A + 1 ml of solution B (mixed well)

(v) **Solution D**
1 ml Folin ciocalteau reagent (Sisco Laboratories, India) + 1 ml DW

11. **10% neutral buffered formalin**

4% formaldehyde in 0.1 M PBS pH 7.4
12. Graded alcohol solutions (v/v) for H&E staining and IHC
20% alcohol: 20 ml absolute alcohol + 80 ml DW
40% alcohol: 40 ml absolute alcohol + 60 ml DW
50% alcohol: 50 ml absolute alcohol + 50 ml DW
60% alcohol: 60 ml absolute alcohol + 40 ml DW
70% alcohol: 70 ml absolute alcohol + 30 ml DW
80% alcohol: 40 ml absolute alcohol + 20 ml DW
90% alcohol: 90 ml absolute alcohol + 10 ml DW

13. Blocking reagents in IHC
Peroxidase blocking agent, 5% \( \text{H}_2\text{O}_2 \): 1.25 ml \( \text{H}_2\text{O}_2 \) + 23.75 ml methanol
Protein blocking agent, 3% SMP: 0.75 gm SMP + 25 ml PBS (0.01 M, pH 7.4)