APPENDIX K

Reagent for tissue fixation and staining

1. Buffered neutral formalin solution (10% neutral formalin)

This buffer contained the folowing ingredients:

37% Formaldehyde solution	100 ml
KH2PO4	4.0 g
K2HPO4	6.5 g

The buffer was prepared by dissolving all the above ingredients in 900 ml of DW

2. Mayer's hematoxylin stain

The stain consisted of the following ingredients:

Hematoxylin crystals	1 g
Sodium iodate	0.2 g
Ammonium or potassium or potassium alum	50 g
Citric acid	1 g
Chloral hydrate	50 g

The stain was prepared by first dissolving the alum in DW and hematoxylin crystals were the added while gently mixing. Other reagents were subsequently added and the final volume was adjusted to 1 L with DW. The solution was stirred gently overnight at 25°C and filtered through a Whatman no. 1 filter paper an kept at 25°C. The final color of the stain should be reddish violet

3. 1% stock alcoholic eosin stain

The stock solution was prepared by dissolving 1 g of Eosin Y (Merck, Darmstadt, Germany) in 20 ml of DW. After completely dissolved, 80 ml of 95% ethanol was added to the preparation. The solution was filtered through a Whatman no. 1 filter paper and kept at 25°C.

The working solution was prepared by adding one part of stock solution into three parts of 80% ethanol then 0.5 ml of glacial acid was added to each 100 ml of the stain.

4. 0.01 M PBS, pH 7.2

To prepare in 1 L, The solution was prepared by dissolving 1.22 g of anhydrous Na_2HPO_4, 0.17 g of anhydrous NaH_2PO_4 and 8.77 g of NaCl in 1 L of DW. The pH of the solution was adjusted to 7.2 with 1 N HCl.

5. Sodium citrate buffer, pH 6.0

5.1 Solution A (0.1 M citric acid)

The solution was prepared by dissolved 21.01 g of citric acid monohydrate in 1 L of DW

5.2 Solution B (0.1 M trisodium citrate)

The solution was prepared by dissolved 29.41 g of sodium citrate tribasic dihydrate in 1 L of DW

The working sodium citrate buffer, pH 6.0 was prepared by mixing 9 ml of solution A with 41 ml of solution B. The pH of this solution was adjusted to 6.0 with 1N HCl and the volume was brought up to 500 ml with DW.