

## Appendices

### **Instrument and Material:**

#### **Instrument:**

Disposable gloves.

Rotary microtome.

Microtome knives.

Coplin jars.

Oven.

Staining racks.

Coated slides.

Water path.

Cover glass.

Dako pen.

Humidity chamber.

#### **Materials:**

Mayer`s haematoxylin.

Xylene.

Ethyl alcohol (absolute, 90%, 70%, 50%).

Distill water.

Peroxidase blocker.

Primary antibody (Hepar-1) .

Secondary antibodies (biotinylated secondary antibody).

3.3 di amino benzidine tetra hydrochloride in substrate buffer.

DPX mounting media.

#### **Phosphate (PH7.4) component:**

**Solution A** (0.2 M sodium di hydrogen orthophosphate, 3.12g di sodium hydrogen orthophosphate, 100 ml DW).

**Solution B** (0.2 M sodium di hydrogen orthophosphate, 2.83g di sodium hydrogen orthophosphate, 100ml DW) (9.5ml from solution A + 40.5ml solution B).

**Citrate buffer(PH6.8) component:**

**Solution A** (0.2 M sodium di hydrogen orthophosphate, 2.83g di sodium hydrogen orthophosphate, 100ml DW).

**Solution B** (2.1g citric acid, 100ml DW) (72.7ml from solution A +22.8ml from solution B).

**Mayer`s haematoxylin component:**

Haematoxylin powder	1 gm
Potassium alum or ammonium alum	50gm
Sodium iodate	0.2gm
Citric acid	1 gm
Chloral hydrate	50gm
Distilled water	1000ml
Ammoniated water:	
Concentrated ammonia	0.05ml
Tap water	99.95ml