APPENDIX 3

PROTEIN PURIFICATION (BUFFERS USED)

Purification of *S. digitata* antigens

List of buffers used in CNBr activation of Sepharose 4 B beads:

1. 2 M Dipotassium hydrogen phosphate, pH 12.0
2. 5 M Dipotassium hydrogen phosphate, pH 12.0
3. 0.1 M Sodium Citrate buffer, pH 6.0
   - 2.45 g citric acid (anhydrous)
   - 10.96 g trisodium citrate dihydrate
   - 8.77 g NaCl (0.15)
   - Add water to 1 liter
4. 3.5% CNBr in 1-2 ml Dimethyl formamide (DMF) to make up the volume with distilled water
5. 0.1 M Carbonate bicarbonate buffer pH 8.7
   - 1.36 g sodium carbonate
   - 7.35 g sodium bicarbonate
   - 950 ml water
   - Adjust pH to 8.7 with 1 M HCl or NaOH
   - Add water to 1 liter
6. 1 M Ethanolamine pH 8.0
   - 61.1 ml ethanolamine, pH 8.0
   - Titrate with HCl to pH 8.0
   - Add water to 1 liter
7. 0.1 M Acetate buffer containing 0.1 M NaCl pH 4.3
   - To 900 ml of water add:
   - 6.80 g Sodium acetate (trihydrate) (0.05 M)
8.77 NaCl (0.15)
Titrate with acetic acid to pH 4.3
Add water to 1 liter.
8. 0.1 M Borate acetate buffer pH 8.6 containing 0.1 M NaCl
9. 0.02 M Tris acetate buffer pH 7.4 containing 0.5 M NaCl
10. 0.02 M Tris buffer pH 7.4 containing 0.1 M NaCl
11. 0.5 M NaCl
12. 3 M Potassium thiocyanate pH 7.4

Purification of *W. bancrofti* recombinant fusion protein

**Cross linking amylose resin**
50mM Glycine-HCl, 0.5 M NaCl pH 2.0
10mM Tris-HCl, pH 7.2

**Column buffer**
10 ml of 0.5 M Sodium phosphate buffer pH 7.2
14.6 g of NaCl
1 ml of 1 M Sodium azide
0.35 ml of 2-mercaptoethanol (final concen. 10 mM)
P pH adjusted to 7.0

**Low salt column buffer**
10 ml of 0.5 M Sodium phosphate buffer pH 7.2
0.87 g NaCl
0.5 ml 1 M Sodium azide
0.35 ml 2-mercaptoethanol
pH adjusted to 7.0