



APPENDIX I

สำนักหอสมุด

1. Reagents for ion-exchange column chromatography (without NaN_3)

1.1 1 M Tris-HCl buffer pH 7.5

Stock Tris-HCl buffer was prepared by dissolving 121 g of Tris-HCl in 900 ml of DW, then pH was adjusted to 7.5 with concentrate HCl. The volume was made up to 1,000 ml and sterilized by autoclaving.

1.2 50 mM Tris-HCl buffer, pH 7.5

To prepared working buffer, 50 ml of stock 1 M Tris-HCl was added to 950 ml of DW, mixed by stirring. This solution was filtered through 0.45 μm Millipore membrane before use.

1.3 0.5 M NaCl –Tris, pH 7.5

This solution was prepared by dissolving 4.38 g of NaCl in 150 ml of 50 mM Tris-HCl buffer, pH 7.5, filtration as described above before use.

1.4 1 M NaCl-Tris, pH 7.5

To prepared this buffer, 58.44 g of NaCl was dissolved in 1,000 ml of 50 mM Tris-HCl buffer, pH 7.5 and filtration as described above.

2. Reagents for anti-E-tag affinity column chromatography

2.1 10x binding buffer (0.2 M phosphate buffer, 0.05% NaN_3 , pH 7.0)

This buffer was prepared by mixing all ingredients:

$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	13.8	g
$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	17.8	g and
NaN_3	0.5	g

The volume was made up to 1,000 ml with DW and the pH was adjusted to 7.0. The preparation was filtered through 0.22 μm Millipore membrane. Working binding buffer was prepared by adding 10 ml of this 10x binding buffer to 90 ml of UDW.

2.2 10x Elution buffer (1.0 M glycine, pH 3.0)

This buffer was prepared by dissolving 75.1 g of glycine in 900 ml of DW. The pH of the solution was adjusted to 3.0 with concentrate HCl and the volume was made up to 1,000 ml with UDW. Sterilization was performed by filtering through 0.22 μm Millipore membrane. Working buffer was prepared by adding 3.0 ml of the 10x elution buffer to 27 ml of UDW.

2.3 1x Neutralizing buffer (1 M Tris, 0.05% NaN_3 , pH 8.2)

To prepare the buffer, 12.1 g of Tris-base and 0.05 g of NaN_3 were dissolved in 80 ml of UDW. The pH was adjusted to 8.2 with concentrate HCl, the volume was made up to 100 ml with DW. Sterilization was performed by 0.22 μm Millipore membrane filtration.

