APPENDIX I

Reagents for HuScFv purification using anti-E-Tag column

1 Reagents for anti-E tag affinity column chromatography

1. Binding buffer (0.2 M phosphate buffer, 0.05% NaN_3, pH7.0) (10X)

This buffer was prepared by mixing all of the following ingredients:

$NaH_2PO_4.H_2O$	13.8	g.
$Na_2HPO_4.2H_2O$	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 a sterile 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. Elution buffer (0.1 M glycine, pH 3.0) (10X)

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 a sterile 0.22 μm Millipore membrane. Working buffer was prepared by adding 3.0 ml of the 10x elution buffer to 27 ml of UDW.

3. Neutralizing buffer (1 M Tris, 0.05% NaN_3, pH 8.2) (1X)

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 filtering through a sterile 0.22 μm Millipore membrane.