

## **APPENDIX C**

**Reagents for indirect ELISA of soluble ScFv (HuScFv-ELISA)****1. Coating buffer (carbonate-bicarbonate buffer, pH 9.6)**

The buffer was prepared by dissolving 1.26 g of NaHCO<sub>3</sub> in 300 ml of DW, then the pH was adjusted to 9.6 with 0.05 Na<sub>2</sub>CO<sub>3</sub> (0.53 g in 100 ml of DW).

**2. PBS, pH 7.4 (1x)**

The solution was prepared by dissolving NaCl (8 g), KCl (0.2 g), Na<sub>2</sub>HPO<sub>4</sub> (1.44 g), and KH<sub>2</sub>PO<sub>4</sub> (0.24 g) in 900 ml of DW. The pH of solution was adjusted to 7.4 with NaOH or HCl and DW was added to 1 litre. The solution was sterilized by autoclave.

**3. Washing solution (PBST)**

Washing solution (PBST) was prepared by mixing Tween-20 in PBS, pH 7.4 to a 0.05 % concentration.

**4. Blocking solution**

The solution was prepared by dissolving 3 g of skim milk (Fraction V, Sigma Chemical Co., St Louis, Minnesota, USA) in 100 ml of 0.01 M PBS, pH 7.4.

**5. Substrate solution (ABTS)**

The substrate solution is ABTS solution (Vector Laboratories, USA) containing 2,2'-azino-bis-(3-benzthiazoline-6-sulfonic acid) and 0.03% H<sub>2</sub>O<sub>2</sub>