700 HARRIS STREET
CHARLOTTESVILLE
VA 22903
U.S.A

TEL: (1) 434 984 2304
FAX: (1) 434 984 2709
WWW.INBIO.COM

**Solutions and reagents for ELISA assays**

1. 50mM carbonate/bicarbonate buffer, pH 9.6:

- Na2CO3 1.59g
- NaHCO3 2.93g
- Dissolve in 1 liter deionized water
- Thimerosal 0.10g/liter (can be added as preservative if necessary).

2. Phosphate buffered saline, pH 7.4, containing 0.05% Tween 20 (PBS-T):

- NaCl 8.00g
- KH2PO4 0.20g
- Na2HPO4 1.15g
- KCl 0.20g
- Thimerosal 0.10g (optional)
- Tween 20 0.5ml

Dissolve in deionized water and bring up to a final volume of 1 liter. To make 1% BSA PBS-T, add 1g bovine serum albumin (heat shocked fraction BSA, Sigma A-7030) to 100ml PBS-T. Filter of discard if 1% BSA PBS-T becomes cloudy.

3. Streptavidin-Peroxidase:

Reconstitute 0.25mg Streptavidin-Peroxidase (Sigma S5512) in 1ml distilled water and store at -20°C in 50µl aliquots. Dilute 1/1000 in 1% BSA PBS-T for use in the assay.

4. Substrate solution, 1mM ABTS in 70mM citrate-phosphate buffer, pH 4.2:

- 70mM citrate-phosphate buffer, pH 4.2
- Solution A = 0.1M anhydrous citric acid, 19.21g/L
- Solution B = 0.2M Dibasic Sodium Phosphate.7$H_2O$, 53.65g/L

For 500ml buffer, mix 147ml A + 103ml B and make up to 500ml with deionized $H_2O$. Add 274mg ABTS to 500ml buffer to make the substrate solution (contains 1mM ABTS).

ABTS = 2,2'-azino-di-(3 ethylbenzthiazoline sulfonic acid), Sigma A1888. The substrate solution is stable at 4°C in the dark. Immediately prior to adding to assay plates, add 1µl 30% $H_2O_2$ solution/ml ABTS. The assay will not work if you do not add the $H_2O_2$