



Solutions and reagents for ELISA

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

- Na₂CO₃ 1.59g
- NaHCO₃ 2.93g
- Dissolve in 1 liter deionized water

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
- Tween-20 0.5ml

Dissolve in deionized water to a final volume of 1 liter.

To make 1% BSA PBS-T, add 1g bovine serum albumin (BSA, Sigma A-7030*) to 100ml PBS-T.

3. Streptavidin-Peroxidase:

Reconstitute 0.25mg Streptavidin-Peroxidase (Sigma S5512) in 1ml distilled water and store at -20°C in 50µl aliquots. Dilute 1/1,000 in 1% BSA PBS-T for use in the assay.

4. Substrate solution, 1mM ABTS in 70mM citrate-phosphate buffer, pH4.2:

- 70mM citrate-phosphate buffer, pH 4.2
- Solution A = 0.1M anhydrous citric acid, 19.21g/L
- Solution B = 0.2M Dibasic Na Phosphate.7H₂O, 53.65g/L

For 500ml buffer, mix 147ml A + 103ml B and make up to 500ml with deionized H₂O. Add 274mg ABTS to 500ml buffer to make the substrate solution (contains 1mM ABTS).

ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-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% H₂O₂ solution/ml ABTS. The assay will not work if you do not add the H₂O₂.

*To prevent buffers from becoming turbid, BSA from Roche Diagnostics (catalog number 0311696400) is recommended for ELISA and MARIA buffer preparations. Buffers that become cloudy should be sterile-filtered or discarded.