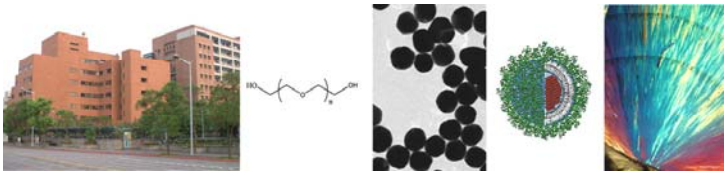
Anti-PEG



Sandwich ELISA assay (AGP4 coating / 3.3-biotin detection)

Materials:

a. EIA plate (Nunc. Maxisorp 430341)

b. Coating buffer (5.3 g Na_2CO3 + 4.2 g $NaHCO_3$ /liter, pH=8.0, adjust pH with 12N HCl)

c. PBS (0.14 M NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.4)

d. Blocking solution: 5% skim milk (Difco #232100) in PBS

e. Dilution buffer: 2% skim milk in PBS.

f. Washing buffer: PBS and PBS-T (PBS containing 0.05% Tween-20)

g. Streptavidin-HRP (Jackson ImmunoResearch #016-030-084)

h. HRP substrate: 50 mg/100 ml ABTS (Sigma #A-1888) in 100 mM phosphate-citrate buffer pH 4.0 (17.4 g K_2HPO_4, 21 g citric acid in 1 Liter Q-H_2O). Immediately before use, add 3 µl of 30% H_2O_2 per 10 ml ABTS substrate solution.

Procedure:

1. Dilute AGP4 to 5 µg/ml in coating buffer. You need 5 ml per microtiter plate.
2. Add 50 µl diluted AGP4 per well and incubate at 37^{o}C for 4 h and then at 4^{o}C overnight.
3. Wash plates 3X with PBS.
4. Add 200 µl blocking solution per well for 2 hours at room temperature.
5. Dilute PEG-compound in dilution buffer to suitable concentrations
6. Wash wells 3X with PBS
7. Add graded concentrations of PEG-compound (50 µl/well) and incubate 2 h at room temperature.
8. Wash with PBS-T 3X and PBS 2X.
9. Add 50 µl/well 3.3-biotin (5 µg/ml in dilution buffer) for 1 h at room temperature.
10. Wash wells with PBS-T 3X and with PBS 2X.
11. Add 50 µl/well streptavidin-HRP (1 µg/ml in dilution buffer) for 1 h at room temperature
12. Wash wells with PBS-T 6X and with PBS 2X.
13. Add 100 µl/well freshly prepared ABTS substrate for 30 min in dark at room temperature.
14. Read absorbance of the wells at 405 nm.

http://www.ibms.sinica.edu.tw/~sroff/anti-PEG/index.html