Anti-PEG

Sandwich ELISA assay (AGP3 coating / AGP3-biotin or AGP4-biotin detection)

Materials:

a. EIA plate (Nunc. Maxisorp 430341)
b. Coating buffer (5.3 g Na₂CO₃ + 4.2 g NaHCO₃ /liter, pH=8.0, adjust pH with 1N NaOH)
c. PBS (0.14 M NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 8.1 mM Na₂HPO₄, 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 K₂HPO₄, 21 g citric acid in 1 Liter Q-H₂O). Immediately before use, add 3 µl of 30% H₂O₂ per 10 ml ABTS substrate solution.

Procedure:

1. Dilute AGP3 to 20 ug/ml in coating buffer. You need 5 ml per microtiter plate.
2. Add 50 µl diluted AGP4 per well and incubate at 37°C for 4 h and then at 4°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. Mix 5 µg/ml AGP4-biotin or AGP3-biotin with 1 µg/ml streptavidin-HRP in dilution buffer.
10. Add 50 µl/well mixture for 1 h at room temperature.
11. Wash wells with PBS-T 6X and with PBS 2X.
12. Add 100 µl/well freshly prepared ABTS substrate for 30 min in dark at room temperature.
13. Read absorbance of the wells at 405 nm.