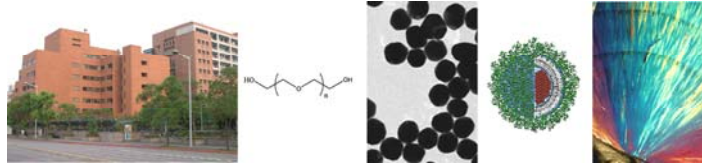


Anti-PEG



Sandwich ELISA assay (rAGP6 coating / 6.3-biotin detection)

Materials:

- EIA plate (Nunc. Maxisorp 430341)
- Coating buffer (5.3 g Na_2CO_3 + 4.2 g NaHCO_3 /liter, pH=8.0, adjust pH with 12N HCl)
- PBS (0.14 M NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4 , 8.1 mM Na_2HPO_4 , pH 7.4)
- Blocking solution: 5% skim milk (Difco #232100) in PBS
- Dilution buffer: 2% skim milk in PBS.
- Washing buffer: PBS and PBS-C (**PBS containing 0.05% CHAPS**)
- Streptavidin-HRP (Jackson ImmunoResearch #016-030-084)
- 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 2 μl of 30% H_2O_2 per 10 ml ABTS substrate solution.

Procedure:

- Dilute rAGP6 to 5 $\mu\text{g}/\text{ml}$ in coating buffer. You need 5 ml per microtiter plate.
- Add 50 μl diluted rAGP6 per well and incubate at 37°C for 4 h and then at 4°C overnight.
- Wash plates 3X with PBS.
- Add 200 μl blocking solution per well for 2 hours at room temperature.
- Wash wells 3X with PBS, plates ready to use.
- Dilute PEG-compound in dilution buffer to suitable concentrations
- Add graded concentrations of PEG-compound (50 $\mu\text{l}/\text{well}$) and incubate 2 h at room temperature.
- Wash with PBS-C 1X with gentle shaking for 5 min and then with PBS 2X for 5 min each.
- Add 50 $\mu\text{l}/\text{well}$ 6.3-biotin (5 $\mu\text{g}/\text{ml}$ in dilution buffer) for 1 h at room temperature.
- Wash with PBS-C 1X with gentle shaking for 5 min and then with PBS 2X for 5 min each.
- Add 50 $\mu\text{l}/\text{well}$ streptavidin-HRP (0.5~1 $\mu\text{g}/\text{ml}$ in dilution buffer) for 1 h at room temperature
- Wash wells with PBS-C 3X, 5 min each and with PBS 2X, 5 min each.
- Add 100 $\mu\text{l}/\text{well}$ freshly prepared ABTS substrate for 30 min in dark at room temperature.
- Read absorbance of the wells at 405 nm.

Note: always prepare fresh coated plates.

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