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AP0002 Protocol

Sandwich ELISA Assay Protocol for Anti-PEG Antibody Pair (AP0002)

Sandwich ELISA Assay Protocol for Anti-PEG Antibody Pair (AP0002):
https://www.novusbio.com/products/polyethylene-glycol-antibody-pair_ap0002
Please centrifuge before opening the vials.

The test protocol is a guideline, user need to determine their optimal experimental condition for best performance.

SECTION 1 Equipments & Reagents

1.1 Anti-PEG Antibody pair
Polyethylene Glycol Matched Antibody Pair (Catalog #: AP0002). This matched antibody pair set binds to the repeating subunits of the polyethylene glycol polymer and can be employed to detect and quantify PEGylated compounds.

1.2 Secondary reagent
Streptavidin-HRP (Jackson ImmunoResearch, Catalog #: 016-030-084)

1.3 Coating buffer (1 Liter)
5.3 g Na2CO3 + 4.2 g NaHCO3, pH=8.0 (adjust pH with 1N NaOH)

1.4 1x PBS
0.14 M NaCl, 2.7 mM KCl, 1.5 mM KH2PO4, 8.1 mM Na2HPO4, pH 7.4

1.5 Blocking solution
5% skim milk in 1x PBS

1.6 Dilution buffer
2% skim milk in 1x PBS

1.7 PBS-T
1x PBS containing 0.2% Tween-20

1.8 ELISA plates
NUNC MaxiSorp(TM) High Protein-Binding Capacity ELISA plates (Catalog #: 44-2404)

1.9 HRP substrate
50 mg/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 ul of 30% H2O2 per 10 ml ABTS substrate solution.

SECTION 2 - Assay Protocol

2.1 Dilute capture antibody to 5 ug/ml in coating buffer.
2.2 Add 50 ul diluted capture antibody per well and incubate at 37C for 4 h and then at 4C overnight.
2.3 Wash plates 3 times with 1x PBS.
2.4 Add 200 ull blocking solution per well for 2 hours at room temperature.
2.5 Dilute PEG-compound (analyte) in dilution buffer to suitable concentrations.
2.6 Wash wells 3 times with 1x PBS.
2.7 Add graded concentrations of PEG-compound (50 ul/well) and incubate 2 h at room temperature.
2.8 Wash with PBS-T 3 times and 1x PBS 2 times.
2.9 Add 50 ul/well detection antibody (5 ug/ml in dilution buffer) for 1 h at room temperature.
2.10 Wash wells with PBS-T 3 times and with 1x PBS 2 times.
2.11 Add 50 ul/well streptavidin-HRP (1 ug/ml in dilution buffer), and incubate for 1 h at room temperature.

2.12 Wash wells with PBS-T 6 times and with 1x PBS 2 times.
2.13 Add 100 ul/well freshly prepared ABTS substrate for 30 min in dark at room temperature.
2.14 Read absorbance of the wells at 405 nm.