

## Sandwich ELISA Assay Protocol for Anti-PEG Antibody Pair

### **!! PLEASE READ BEFORE USE !!**

- ◆ 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 #: AP0001). 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 Na<sub>2</sub>CO<sub>3</sub> + 4.2 g NaHCO<sub>3</sub>, pH=8.0 (adjust pH with 1N NaOH)

### **1.4 1x PBS**

0.14 M NaCl, 2.7 mM KCl, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 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™ 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 K<sub>2</sub>HPO<sub>4</sub>, 21 g citric acid in 1 Liter Q-H<sub>2</sub>O). Immediately before use, add 3 µl of 30% H<sub>2</sub>O<sub>2</sub> per 10 ml ABTS substrate solution.

## **SECTION 2 - Assay Protocol**

2.1 Dilute capture antibody to 5 µg/ml in coating buffer.

2.2 Add 50 µl diluted capture antibody per well and incubate at 37°C for 4 h and then at 4°C overnight.

- 2.3 Wash plates 3 times with 1x PBS.
- 2.4 Add 200  $\mu$ l 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  $\mu$ l/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  $\mu$ l/well detection antibody (5  $\mu$ g/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  $\mu$ l/well streptavidin-HRP (1  $\mu$ g/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  $\mu$ l/well freshly prepared ABTS substrate for 30 min in dark at room temperature.
- 2.14 Read absorbance of the wells at 405 nm.