RevMAb Biosciences

ELISA Protocol for RevMAb Matched Antibody Pair

Reagents

1. Capture Antibody: Rabbit Monoclonal Antibody, 100 ug at 1.0 mg/mL in 50% Glycerol/PBS with 1% BSA and 0.09% sodium azide;
2. Detection Antibody: Biotin Rabbit Monoclonal Antibody, 25 ug at 1.0 mg/mL in 50% Glycerol/PBS with 1% BSA and 0.09% sodium azide;
3. 10X Sample Diluent.
4. Coating Buffer: 0.2 M Sodium Carbonate-Bicarbonate buffer pH9.4.
5. Blocking buffer: 20mM Tris-HCl, 0.15M NaCl (TBS) containing 0.05% v/v Tween®-20 and 1% w/v BSA.
6. Wash buffer: 20mM Tris-HCl, 0.15M NaCl (TBS) containing 0.05% v/v Tween®-20.

Method

1. Coat microtiter plate wells with 100 µL/well of Capture Antibody at 0.5-2 µg/ml in coating buffer. Cover the plate and incubate overnight at 4°C or 2 hours at 37°C. Wash the plate 3 times in wash buffer.
2. Add 150-200 µl/well of blocking buffer. Incubate for 1-2 hours at room temperature. Discard the blocking buffer.
3. Dilute samples in Sample Diluent, and then add 100 µL/well of diluted samples to microtiter plate. Incubate for 1-2 hours at room temperature. Wash 3 times in wash buffer.
4. Add 100 µl/well of biotin-conjugated detection antibody (0.1-0.5 µg/ml in blocking buffer). Incubate for 1-2 hour at room temperature. Wash 3 times in wash buffer.
5. Add 100 µl/well of enzyme-conjugated streptavidin (appropriately diluted in blocking buffer). Incubate for 1 hour at room temperature. Wash 3 times in wash buffer.
6. Add 100 µl/well of the appropriate substrate solution. Incubate at room temperature (and in the dark if required) for appropriate time until desired color change is attained. Add appropriate stop solution to each well.
7. Read absorbance values at the appropriate wavelength.