ELISA: Sandwich TMB Protocol

Standard and Antibody

PeproTech's Recombinant Protein[1]
PeproTech's Antigen Affinity Purified Polyclonal or Monoclonal Antibody[1]
PeproTech's Biotinylated Antigen Affinity Purified Polyclonal Antibody[1]
**PeproTech's Standards and Antibodies should be reconstituted according to the data sheets which accompany each product.

Recommended Materials

ELISA microplates (Nunc MaxiSorp Prod. # 439454, or Corning Prod. # 3590)[2];
Tween-20 (Sigma Cat. # P-7949);
BSA (Sigma Cat. # A-7030);
Streptavidin-HRP conjugate (Pierce Cat. # 31032)[1];
TMB Liquid Substrate Solution (KPL Cat. # 52-00-02)[2];
Dulbecco's PBS [10x] (Gibco BRL Cat. #14200-075)
Sealing Film[2].

Recommended Solutions

All solutions should be at ambient temperature prior to use.
PBS: Dilute 10xPBS to 1xPBS, pH 7.20 in sterile water[2].
Wash Buffer: 0.05% Tween-20 in PBS[2]
Block Buffer: 1% BSA in PBS*[2]
Diluent: 0.05% Tween-20, 0.1% BSA in PBS*[2]

*Sterile filter and store at 4°C for up to 1 week.

[1]Included in Standard and Mini ELISA Development Kits
[2]Included in ELISA Buffer Kit (900-T00)

Plate Preparation

1. Dilute capture antibody (polyclonal) with PBS to a concentration of 1µg/ml. Immediately, add 100µl to each ELISA plate well. Seal the plate and incubate overnight at room temperature. (Monoclonal Antibody – at least 2 µg/ml).
2. Aspirate the wells to remove liquid and wash plates 4 times. Each wash consists of adding 300µl wash buffer per well, followed by aspiration. After the last wash invert plate to remove residual buffer and blot on paper towel.
3. Add 300µl blocking buffer to each well. Incubate 1 hour at R.T.
4. Aspirate and wash plate 4 times (as in step 2).

ELISA Protocol

Standard/Sample: Serial dilute standard from 0.01µg/ml to zero in diluent. Add 100µl of standard or sample to each well in triplicate. Incubate at room temperature for at least 2 hours.

Detection: Wash plate four times. Dilute detection antibody (biotinylated) in diluent to a concentration of 0.5µg/ml (500ng/ml). Immediately add 100µl per well. Incubate at room temperature for 2 hours.

Streptavidin-HRP Conjugate: Aspirate and wash plate 4 times. Dilute Streptavidin-HRP conjugate 1:20,000 in diluent. Add 100µl per well. Incubate 30 min at room temperature. (Dilutions can also be tried at 1:10,000 and 1:40,000, though a dilution of 1:20,000 is most commonly used.)

TMB Liquid Substrate: Aspirate and wash plate 4 times. Add 100µl of substrate solution to each well. Incubate at room temperature for 20 minutes. Add 100µl 1M HCl stop solution to each well. Monitor color development with an ELISA plate reader at 450 nm with wavelength correction set at 620 nm.

Note: Reliable standard curves are obtained when O.D. readings do not exceed 0.150 units for the zero standard concentrations. Development time and O.D. readings may vary.