# ELISA: Direct Protocol

## Standard and Antibody

PeproTech's Recombinant Protein[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);
Avidin-HRP conjugate (Sigma Cat. # A-7419)[1];
ABTS Liquid Substrate Solution (Sigma Cat. # A3219)[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 (Cat. # 900-K00)*

## Plate Preparation

1. Standard/Sample: Serial dilute standard from 0.1µg/ml to zero in PBS. Add 100µl of standard or sample to each well in triplicate. Incubate at room temperature overnight.
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 2 hour at R.T.
4. Aspirate and wash plate 4 times (as in step 2).

## ELISA Protocol

Detection: Wash plate four times. Dilute detection antibody (biotinylated) in diluent to a concentration of 1 µg/ml. Immediately add 100µl per well. Incubate at room temperature for 2 hours.

Avidin-HRP Conjugate: Aspirate and wash plate 4 times. Dilute Avidin-HRP conjugate 1:2000 in diluent.
Add 100µl per well. Incubate 30 min at room temperature.

Substrate: Aspirate and wash plate 4 times. Add 100µl of substrate solution to each well. Incubate at room temperature for color development. Monitor color development with an ELISA plate reader at 405 nm with wavelength correction set at 650 nm.

Note: Reliable standard curves are obtained when O.D. readings do not exceed 0.2 units for the zero standard concentrations, or 1.2 units for the highest standard concentration. The plate should be monitored at 5 minute intervals until desired O.D. readings are obtained. The typical range is 5-40 minutes. O.D. readings may vary.

*Assay sensitivity may be increased with additional washings.*