ReliaTech
Receptor Ligand Technologies GmbH

ELISA: Sandwich ABTS

A. STANDARD AND ANTIBODY

- ReliaTech´s Recombinant Protein
- ReliaTech´s Biotinylated Affinity Purified Polyclonal Antibody
- ReliaTech´s Affinity Purified Polyclonal or Monoclonal Antibody

B. RECOMMENDED MATERIALS

- ELISA microplates
- Tween-20
- BSA
- Avidin-HRP conjugate
- ABTS Liquid Substrate Solution
- Dulbecco´s PBS (10x)
- Sealing film

C. RECOMMENDED SOLUTIONS

- PBS: Dilute 10x PBS to 1x PBS, pH 7.2 in sterile water
- Wash Buffer: 0.05% Tween-20 in PBS, 0.2 µm-filtration
- Block Buffer: 1% BSA in PBS, 0.2 µm-filtration
- Diluent: 0.05% Tween-20, 0.1% BSA in PBS

All solutions should be at ambient temperature prior to use.

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1. PLATE PREPARATION

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

2. ELISA

1. Standard/Sample: Serial dilute standard from 0.1 µg/mL to 0.0 µg/mL in PBS. Add 100 µL of standard or sample to each well in triplicate. Incubate at room temperature overnight (at least 3 hours).
2. Detection: Wash plate 4 times. Dilute detection antibody (biotinylated) in diluent to a concentration of 0.5 µg/mL. Immediately add 100 µL per well. Incubate at room temperature for 2 hours.
3. Avidin-HRP Conjugate: Aspirate and wash plate 4 times. Dilute Avidin-HRP conjugate 1:2000 in diluent. Add 100 µL per well. Incubate 30 min. at room temperature.

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

   Reliable standard curves are obtained when OD readings do not exceed 0.2 units for the 0 standard concentrations, or 1.2 units for the highest standard concentration. The plate should be monitored at 5 minute intervals until desired OD readings are obtained. Typical range is 5-40 minutes. OD readings may vary.

   Assay sensitivity may be increased with additional washings.