

Product Information

Sigma's Sandwich ELISA kits are *in vitro* enzyme-linked immunosorbent assays for the quantitative measurement of soluble proteins in a variety of species. Our extensive ELISA selection includes cytokines, growth factors, proteases, soluble receptors, apoptosis effectors, and many other soluble proteins.

Sandwich Assay Procedure

1. Bring all reagents and samples to room temperature (18–25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Add 100 μ L of each standard and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature or overnight at 4 °C with gentle shaking.

3. Discard the solution and wash 4 times with 1 \times Wash Solution. Wash by filling each well with Wash Buffer (300 μ L) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 μ L of 1 \times prepared Detection Antibody to each well. Cover wells and incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash procedure as in step 3.

6. Add 100 μ L of prepared Streptavidin solution to each well. Cover wells and incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution. Repeat the wash as in step 3.

8. Add 100 μ L of TMB One-Step Substrate Reagent (Item H) to each well. Cover wells and incubate for 30 minutes at room temperature in the dark with gentle shaking.

9. Add 50 μ L of Stop Solution (Item I) to each well. Read at 450 nm immediately.

How It Works

