Assay Procedure

1. Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.
2. Prepare all reagents, standard dilutions, and samples as directed in the product insert.
3. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
4. Add 100 µL 1x Assay diluent to the blank wells. Add 100 µl of each standard and samples into the designated wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4ºC with gentle shaking.
5. Decant or aspirate contents of wells. Wash wells by filling with at least 300 µl/well prepared wash buffer followed by decanting/aspirating. Soak wells in wash buffer for 30 seconds to 1 minute for each wash. Repeat wash 4 times for a total of 5 washes. After the last wash, blot plate on absorbent paper to remove residual buffer. Thorough washing at this step is very important, complete removal of liquid is required for proper performance.
6. Add 100 µl of 1X prepared biotinylated antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 4.
8. Add 100 µl of prepared Streptavidin solution to each well. Incubate for 45 minutes at room temperature with gentle shaking.
9. Discard the solution. Repeat the wash as in step 4.
10. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
11. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately. Read absorbance at 450nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract the optical density readings at 570nm from readings at 450nm.