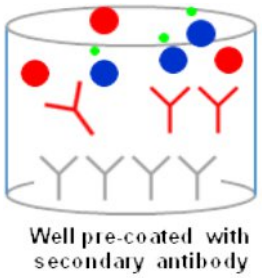
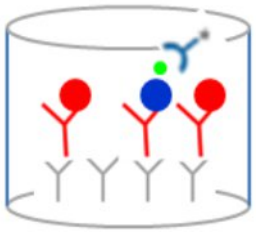
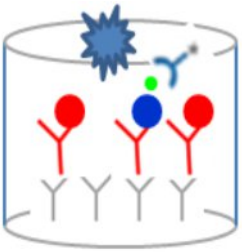
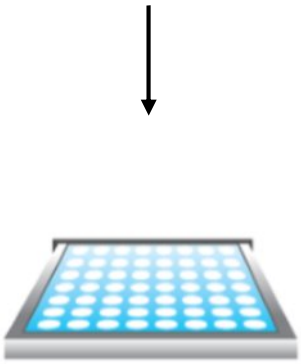
Assay Procedure

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. Pipette 25 µl of Primary antibody into all wells.
5. Pipette 75 ul of 1x assay diluent into the blank wells and 50 µl of 1x assay diluent into the Bo (0 ng/ml standard) wells. Pipette 50 µl of Standards into the standard wells and 50 µl of samples into the sample wells.
6. Pipette 25 µl of biotinylated peptide into each well except the Blank wells.
7. Seal the plate. Incubate for 1~1.5 hours at room temperature with gentle shaking.
8. Decant each well and wash. Repeating the process 4 times for a total of 5 washes.
9. Pipette 100 µl of diluted streptavidin-HRP solution into each well.









10. Seal the plate. Incubate for 45 min at room temperature with gentle shaking.
11. Wash plate as above (Step 5).
12. Add 100 µL Substrate Solution to each well. Incubate at room temperature for 15 minutes. PROTECT FROM LIGHT
13. Add 50 µL of Stop Solution to each well. Read at 450 nm within 30 minutes. Set wavelength correction to 570 nm.