## Protocol

(Below is a sample, please refer to the manual included in the kit)

### REAGENT PREPARATION

1. Bring all kit components and samples to room temperature before use.
2. Bring microtiter plate to room temperature before opening. Remove the desired number of well strips and immediately reseal and store at 2-8° C.
3. Dispense5 μL of BALANCE SOLUTION into 50 μL experimental samples.

NOTE: This step is required only when the sample is cell culture supernatant, body fluid or tissue homogenate.

4. Dilute 10 mL of WASH SOLUTION concentrate (100×) with 990 mL of deionized or distilled water. If crystals have formed in the concentrate warm to room temperature and mix to dissolve.

### ASSAY PROCEDURE

1. Add 50 μL of SAMPLE or STANDARD to the appropriate wells in the supplied microtiter plate. Note that wells have been pre-blocked and no additional blocking steps are required.
2. Incubate 1 hour at room temperature.
3. Empty wells and wash 3-5 times with 300-400 μL 1X WASH SOLUTION per well.
4. Empty final wash and add 100 μL of CONJUGATE per well and mix well. Cover and incubate 1 hour at 37° C in a humid chamber.
5. Wash each well 5 times with 1X WASH SOLUTION. After the last wash invert the plate and blot dry by tapping on absorbent paper. Note: Hold the sides of the plate frame firmly when washing to assure that all strips remain securely in the frame. Complete removal of the liquid at each step is essential for good performance.
6. Add 50 μL SUBSTRATE A to each well followed by addition of 50 μL SUBSTRATE B. Cover and incubate 10-15 minutes at room temperature. SUBSTRATE is light sensitive. Keep out of direct sunlight or cover with foil.
7. Add 50 μL of STOP SOLUTION to each well. Mix well.
8. Immediately read the optical density (O.D.) at 450 nm.
9. Subtract the mean blank value from each SAMPLE or STANDARD value and calculate the mean for duplicate (or greater) wells.
10. Construct the standard curve using graph paper or statistical software.