SAMPLE PROTOCOL FOR NO WASH CAPTURE SANDWICH IMMUNOASSAY

Microspheres should be protected from prolonged exposure to light throughout this procedure.

1. Select the appropriate antibody-coupled microsphere sets.

2. Resuspend the microspheres by vortex and sonication for approximately 20 seconds.

3. Prepare a Working Microsphere Mixture by diluting the coupled microsphere stocks to a final concentration of 200 microspheres of each set/μL in PBS-1% BSA. (Note: 25 μL of Working Microsphere Mixture is required for each reaction.) See **Technical Note 1**.

4. Aliquot 25 μL of the Working Microsphere Mixture to the appropriate wells of a round-bottom microtiter plate.

5. Add 25 μL of PBS-1%BSA to each background well.

6. Add 25 μL of standard or sample to the appropriate wells.

7. Mix the reactions gently by pipetting up and down several times with a multi-channel pipettor.

8. Cover the plate and incubate for 30 minutes at room temperature.

9. Dilute the biotinylated detection antibody to the appropriate concentration in PBS-1% BSA. (Note: 25 μL of diluted detection antibody is required for each reaction). See **Technical Note 2**.

10. Add 25 μL of the diluted detection antibody to each well.

11. Mix the reactions gently by pipetting up and down several times with a multi-channel pipettor.

12. Cover the plate and incubate for 30 minutes at room temperature.

13. Dilute streptavidin-R-phycoerythrin to the appropriate concentration in PBS-1% BSA. (Note: 25 μL of diluted streptavidin-R-phycoerythrin is required for each reaction). See **Technical Note 2**.

14. Add 25 μL of the diluted streptavidin-R-phycoerythrin to each well.

15. Mix the reactions gently by pipetting up and down several times with a multi-channel pipettor.

16. Cover the plate and incubate for 15-30 minutes at room temperature.

17. OPTIONAL – Include the following steps if high backgrounds occur:

- **a.** Pre-wet a 1.2 μm Millipore filter plate with 100 μL/well of PBS-1% BSA and aspirate by vacuum manifold.
- **b.** Add 50 μL of PBS-1% BSA to the appropriate wells of the filter plate.
- **c.** Transfer the contents of round-bottom plate to the filter plate.
- **d.** Aspirate the supernatant by vacuum manifold.
- **e.** Wash each well twice with 100 μL of PBS-1% BSA and aspirate by vacuum manifold.
- **f.** Resuspend the microspheres in 100 μL of PBS-1% BSA by gently pipetting up and down five times with a multi-channel pipettor.

18. Analyze 50-75 μL on the Luminex analyzer according to the system manual.

Technical Note 1: Either PBS-1% BSA or PBS-BN (PBS, 1% BSA, 0.05% Azide, pH 7.4) may be used as Assay Buffer.

Technical Note 2: For a no-wash assay, the concentration of the detection antibody and the streptavidin-R-phycoerythrin should be optimized. The optimal concentrations tend to be higher than in a washed assay.