SAMPLE PROTOCOL FOR WASHED CAPTURE SANDWICH IMMUNOASSAY USING MAGNETIC MICROSPHERES

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 100 microspheres of each set/µL in PBS-1% BSA. (Note: 50 µL of Working Microsphere Mixture is required for each reaction.) See **Technical Note 1**.

4. Aliquot 50 µL of the Working Microsphere Mixture into the appropriate wells of a round-bottom plate.

5. Add 50 µL of PBS-1% BSA to each background well.

6. Add 50 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 on a plate shaker set to approximately 800 rpm.

9. Place the plate into the magnetic separator and allow separation to occur for 30 to 60 seconds. See **Technical Note 2**.

10. Use a multi-channel pipette to carefully aspirate the supernatant from each well. Take care not to disturb the microspheres.

11. Leave the plate in the magnetic separator for the following wash steps:
- **a.** Add 100 µL PBS-1% BSA to each well.
- **b.** Use a multi-channel pipette to carefully aspirate the supernatant from each well. Take care not to disturb the microspheres.
- **c.** Repeat steps **a.** and **b.** above.

12. Remove the plate from the magnetic separator and resuspend the microspheres in 50 µL of PBS-1% BSA by gently pipetting up and down several times using a multi-channel pipettor.

13. Dilute the biotinylated detection antibody to 4 µg/mL in PBS-1% BSA. (Note: 50 µL of diluted detection antibody is required for each reaction.) See **Technical Note 3**.

14. Add 50 µL of the diluted detection antibody 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 30 minutes at room temperature on a plate shaker set to approximately 800 rpm.

17. Place the plate into the magnetic separator and allow separation to occur for 30 to 60 seconds.

18. Use a multi-channel pipette to carefully aspirate the supernatant from each well. Take care not to disturb the microspheres.

19. Leave the plate in the magnetic separator for the following wash steps:
    - **a.** Add 100 µL PBS-1% BSA to each well.
    - **b.** Use a multi-channel pipette to carefully aspirate the supernatant from each well. Take care not to disturb the microspheres.
    - **c.** Repeat steps **a.** and **b.** above.

20. Remove the plate from the magnetic separator and resuspend the microspheres in 50 µL of PBS-1% BSA by gently pipetting up and down several times with a multi-channel pipettor.

21. Dilute streptavidin-R-phycoerythrin reporter to 4 µg/mL in PBS-1% BSA. (Note: 50 µL of diluted streptavidin-R-phycoerythrin is required for each reaction.) See **Technical Note 3**.

22. Add 50 µL of the diluted streptavidin-R-phycoerythrin to each well.

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

24. Cover the plate and incubate for 30 minutes at room temperature on a plate shaker set to approximately 800 rpm.

25. Place the plate into the magnetic separator and allow separation to occur for 30 to 60 seconds.

26. Use a multi-channel pipette to carefully aspirate the supernatant from each well. Take care not to disturb the microspheres.

27. Leave the plate in the magnetic separator for the following wash steps:
    - **a.** Add 100 µL PBS-1% BSA to each well.
    - **b.** Use a multi-channel pipette to carefully aspirate the supernatant from each well. Take care not to disturb the microspheres.
    - **c.** Repeat steps **a.** and **b.** above.

28. Remove the plate from the magnetic separator and resuspend the microspheres in 100 µL of PBS-1% BSA by gently pipetting up and down several times with a multi-channel pipettor.

29. 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 list of magnetic separator plates, see **Recommended Materials for Magnetic Microspheres**. Optimal separation time may vary with the type of separator used.

Technical Note 3: Concentrations should be optimized for specific reagents, assay conditions, level of multiplexing, etc. in use.