

## SAMPLE PROTOCOL FOR WASHED 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 100 microspheres of each set/ $\mu\text{L}$  in PBS-1% BSA. (Note: 50  $\mu\text{L}$  of Working Microsphere Mixture is required for each reaction.) See **Technical Note 1**.
4. Pre-wet a 1.2  $\mu\text{m}$  Millipore filter plate with 100  $\mu\text{L}$ /well of PBS-1% BSA and aspirate by vacuum manifold.
5. Aliquot 50  $\mu\text{L}$  of the Working Microsphere Mixture into the appropriate wells of the filter plate.
6. Add 50  $\mu\text{L}$  of PBS-1% BSA to each background well.
7. Add 50  $\mu\text{L}$  of standard or sample to the appropriate wells.
8. Mix the reactions gently by pipetting up and down several times with a multi-channel pipettor.
9. Cover the filter plate and incubate for 30 minutes at room temperature on a plate shaker.
10. Aspirate the supernatant by vacuum manifold.
11. Wash each well twice with 100  $\mu\text{L}$  of PBS-1% BSA and aspirate by vacuum manifold.
12. Resuspend the microspheres in 50  $\mu\text{L}$  of PBS-1% BSA by gently pipetting up and down five times with a multi-channel pipettor.
13. Dilute the biotinylated detection antibody to 4  $\mu\text{g}/\text{mL}$  in PBS-1% BSA. (Note: 50  $\mu\text{L}$  of diluted detection antibody is required for each reaction.) See **Technical Note 2**.
14. Add 50  $\mu\text{L}$  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 filter plate and incubate for 30 minutes at room temperature on a plate shaker.
17. Aspirate the supernatant by vacuum manifold.
18. Wash each well twice with 100  $\mu\text{L}$  of PBS-1% BSA and aspirate by vacuum manifold.
19. Resuspend the microspheres in 50  $\mu\text{L}$  of PBS-1% BSA by gently pipetting up and down five times with a multi-channel pipettor.

20. Dilute streptavidin-R-phycoerythrin reporter to 4  $\mu\text{g}/\text{mL}$  in PBS-1% BSA. (Note: 50  $\mu\text{L}$  of diluted streptavidin-R-phycoerythrin is required for each reaction.) See **Technical Note 2**.
21. Add 50  $\mu\text{L}$  of the diluted streptavidin-R-phycoerythrin to each well.
22. Mix the reactions gently by pipetting up and down several times with a multi-channel pipettor.
23. Cover the filter plate and incubate for 30 minutes at room temperature on a plate shaker.
24. Aspirate the supernatant by vacuum manifold.
25. Wash each well twice with 100  $\mu\text{L}$  of PBS-1% BSA and aspirate by vacuum manifold.
26. Resuspend the microspheres in 100  $\mu\text{L}$  of PBS-1% BSA by gently pipetting up and down five times with a multi-channel pipettor.
27. Analyze 50-75  $\mu\text{L}$  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:** Concentrations should be optimized for specific reagents, assay conditions, level of multiplexing, etc. in use.