SAMPLE PROTOCOL FOR WASHED SEROLOGICAL ASSAY USING MAGNETIC MICROSPHERES

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

1. Dilute samples and controls using diluent (for example, dilute 1 to 500).

2. Select the appropriate antigen-coupled microsphere mixture at working concentration.

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

4. Aliquot 50 µL of the working microsphere mixture into the appropriate wells of a white round-bottom plate.

5. Add 50 µL of diluted controls and diluted samples to the appropriate wells (see example plate layout below).

6. If available, add 50 µL of standard to the appropriate wells. (Note: Many standards are supplied prediluted at working concentration and do not require further dilution).

7. Cover the plate to protect it from light and incubate for 60 minutes at room temperature on a plate shaker set to approximately 800 rpm.

8. Place the plate into the magnetic separator and allow separation to occur for 60 seconds. See **Technical Note 1**.

9. Carefully remove the supernatant from each well using the manual inversion wash method. Take care not to disturb the microspheres. See **Technical Note 2.**

10. Leave the plate in the magnetic separator for the following wash steps:

- **a.** Add 100 µL of wash buffer to each well.
- **b.** Carefully remove the supernatant from each well using the manual inversion wash method. Take care not to disturb the microspheres.
- **c.** Repeat steps **a.** and **b.** above.

11. Remove the plate from the magnetic separator and add 100 µL of detection antibody to each well of the plate.

12. Cover the plate to protect it from light and incubate for 30 minutes at room temperature on a plate shaker set to approximately 800 rpm.

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

14. Carefully remove the supernatant from each well using the manual inversion wash method. Take care not to disturb the microspheres.

15. Leave the plate in the magnetic separator for the following wash steps:

a. Add 100 µL of wash buffer to each well.
b. Carefully remove the supernatant from each well using the manual inversion wash method. Take care not to disturb the microspheres.
c. Repeat steps **a.** and **b.** above.

16. Remove the plate from the magnetic separator and add 100 µL of reporter conjugate (e.g SA-PE) to each well of the plate.

17. Cover the plate to protect it from light and incubate for 30 minutes at room temperature on a plate shaker set to approximately 800 rpm.

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

19. Carefully remove the supernatant from each well using the manual inversion wash method. Take care not to disturb the microspheres.

20. Leave the plate in the magnetic separator for the following wash steps:

a. Add 100 µL of wash buffer to each well.
b. Carefully remove the supernatant from each well using the manual inversion wash method. Take care not to disturb the microspheres.
c. Repeat steps **a.** and **b.** above.

21. Remove the plate from the magnetic separator and add 100 µL of wash buffer to each well of the plate.

22. Resuspend the microspheres by pipetting up and down several times with a multi-channel pipettor or placing the plate onto a plate shaker for approximately 15 seconds.

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

24. Analyze 75 µL on the Luminex analyzer according to the system manual.

Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Neg (clear)	S4	samples									
B	Neg (clear)	S4	↓									
C	Pos	S3										
D	Pos	S3										
E	HPos	S2										
F	HPos	S2										
G	S5	S1										
H	S5	S1										

Plate 2 **(and all others within multi-plate batch)**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Neg (clear)											
B	Neg (clear)											
C	Pos											
D	Pos											
E	HPos											
F	HPos											
G	samples											
H	↓											

Example Plate Layouts

Technical Note 1: For a list of magnetic separator plates, see **Recommended Materials for Magnetic Microspheres**:
http://www.luminexcorp.com/uploads/data/Magnetic%20Microspheres%20FAQs/Recommended%20Materials%20for%20Magnetic%20Microspheres%200207%2011420.pdf

Optimal separation time may vary with the type of separator used.

Technical Note 2: Manual wash method instuctions can be found at:
http://www.luminexcorp.com/support/Magnetic%20Microspheres/Washing_Method_whitepaper.pdf