SAMPLE PROTOCOL FOR TWO-STEP CARBODIIMIDE COUPLING OF PROTEIN TO MagPlex™-C MAGNETIC CARBOXYLATED MICROSPHERES

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

1. Resuspend the stock uncoupled microsphere suspension according to the instructions described in the Product Information Sheet provided with your microspheres.

2. Transfer 5.0×10^6 of the stock microspheres to a **USA Scientific** microcentrifuge tube.

3. Place the tube into a magnetic separator and allow separation to occur for 30 to 60 seconds. See **Technical Note 1**.

4. With the tube still positioned in the magnetic separator, remove the supernatant. Take care not to disturb the microspheres.

5. Remove the tube from the magnetic separator and resuspend the microspheres in 100 µL dH_2O by vortex and sonication for approximately 20 seconds.

6. Place the tube into a magnetic separator and allow separation to occur for 30 to 60 seconds.

7. With the tube still positioned in the magnetic separator, remove the supernatant. Take care not to disturb the microspheres.

8. Remove the tube from the magnetic separator and resuspend the washed microspheres in 80 µL 100 mM Monobasic Sodium Phosphate, pH 6.2 by vortex and sonication for approximately 20 seconds.

9. Add 10 µL of 50 mg/mL Sulfo-NHS (diluted in dH_20) to the microspheres and mix gently by vortex.

10. Add 10 µL of 50 mg/mL EDC (diluted in dH_20) to the microspheres and mix gently by vortex.

11. Incubate for 20 minutes at room temperature with gentle mixing by vortex at 10 minute intervals.

12. Place the tube into a magnetic separator and allow separation to occur for 30 to 60 seconds.

13. With the tube still positioned in the magnetic separator, remove the supernatant. Take care not to disturb the microspheres.

14. Remove the tube from the magnetic separator and resuspend the microspheres in 250 µL of 50 mM MES, pH 5.0 by vortex and sonication for approximately 20 seconds. See **Technical Note 2**.

15. Repeat steps 12. and 13. This is a total of two washes with 50 mM MES, pH 5.0.

16. Remove the tube from the magnetic separator and resuspend the activated and washed microspheres in 100 µL of 50 mM MES, pH 5.0 by vortex and sonication for approximately 20 seconds.

17. Add 125, 25, 5 or 1 µg protein to the resuspended microspheres. (Note: We recommend titration in the 1 to 125 µg range to determine the optimal amount of protein per specific coupling reaction.)

18. Bring total volume to 500 µL with 50 mM MES, pH 5.0.

19. Mix coupling reaction by vortex.

20. Incubate for 2 hours with mixing (by rotation) at room temperature.

21. Place the tube into a magnetic separator and allow separation to occur for 30 to 60 seconds.

22. With the tube still positioned in the magnetic separator, remove the supernatant. Take care not to disturb the microspheres.

23. Remove the tube from the magnetic separator and resuspend the coupled microspheres in 500 µL of PBS-TBN by vortex and sonication for approximately 20 seconds. See **Technical Note 3**.

24. Incubate for 30 minutes with mixing (by rotation) at room temperature. (Note: Optional – perform this step when using the microspheres the same day.)

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

26. With the tube still positioned in the magnetic separator, remove the supernatant. Take care not to disturb the microspheres.

27. Remove the tube from the magnetic separator and resuspend the microspheres in 1 mL of PBS-TBN by vortex and sonication for approximately 20 seconds. See **Technical Note 4**.

28. Repeat steps 25. and 26. This is a total of two washes with 1 mL PBS-TBN.

29. Remove the tube from the magnetic separator and resuspend the coupled and washed microspheres in 250-1000 µL of PBS-TBN.

30. Count the microsphere suspension by hemacytometer.

Calculation: Total microspheres = count (1 corner of 4 x 4 section) x (1 x 10^4) x (dilution factor) x (resuspension volume in mL)

31. Store coupled microspheres refrigerated at 2-8°C in the dark.

Technical Note 1: For a list of magnetic separators, see **Recommended Materials for Magnetic Microspheres**. Optimal separation time may vary with the type of separator used.

Technical Note 2: Coupling can be performed in 100 mM MES, pH 6.0 with similar results. For some proteins, better solubility and better coupling may be achieved at a higher coupling pH or in a different buffer. If your protein does not couple satisfactorily under these recommendations, try PBS, pH 7.4 as an alternate coupling buffer.

Technical Note 3: Either PBS-TBN (PBS, 0.1% BSA, 0.02% Tween-20, 0.05% Azide, pH 7.4) or PBS-BN (PBS, 1% BSA, 0.05% Azide, pH 7.4) may be used as Blocking/Storage Buffer.

Technical Note 4: Either PBS-TBN or PBS, 0.05% Tween-20 may be used as Wash Buffer.