SAMPLE PROTOCOL FOR CARBODIIMIDE COUPLING OF AMINE-MODIFIED OLIGONUCLEOTIDES TO MagPlex™-C MAGNETIC CARBOXYLATED MICROSPHERES

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

1. Bring a fresh aliquot of -20°C, desiccated **Pierce** EDC powder to room temperature.

2. Resuspend the amine-substituted oligonucleotide ("probe" or "capture" oligo) to 1 mM (1 nanomole/μL) in **dH_2O.**

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

4. Transfer 5.0 x 10^6 of the stock microspheres to a **USA Scientific** microfuge tube.

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

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

7. Remove the tube from the magnetic separator and resuspend the microspheres in 50 μL of 0.1 M MES, **pH 4.5** by vortex and sonication for approximately 20 seconds.

8. Prepare a 1:10 dilution of the 1 mM capture oligo in **dH_2O** (0.1 nanomole/μL).

9. Add 2 μL (0.2 nanomole) of the 1:10 diluted capture oligo to the resuspended microspheres and mix by vortex. See **Technical Note 2**.

10. Prepare a fresh solution of 10 mg/mL EDC in **dH_2O**. (Note: Return the EDC powder to desiccant to re-use for the second EDC addition.)

11. One by one for each coupling reaction, add 2.5 μL of fresh 10 mg/mL EDC to the microspheres (25 μg or ≅ $[0.5\ \mu g/\mu L]_{final}$) and mix by vortex.

12. Incubate for 30 minutes at room temperature in the dark.

13. Prepare a second fresh solution of 10 mg/mL EDC in dH_2O. (Note: The aliquot of EDC powder should now be discarded. We recommend using a fresh aliquot of EDC powder for each coupling episode.)

14. One by one for each coupling reaction, add 2.5 μL of fresh 10 mg/mL EDC to the microspheres and mix by vortex.

15. Incubate for 30 minutes at room temperature in the dark.

16. Add 1.0 mL of 0.02% Tween-20 to the coupled microspheres.

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

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

19. Remove the tube from the magnetic separator and resuspend the coupled microspheres in 1.0 mL of 0.1% SDS by vortex.

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

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

22. Remove the tube from the magnetic separator and resuspend the coupled microspheres in 100 µL of TE, pH 8.0 by vortex and sonication for approximately 20 seconds.

23. Enumerate the coupled microspheres by hemacytometer:

- **a.** Dilute the resuspended, coupled microspheres 1:100 in dH_20.
- **b.** Mix thoroughly by vortex.
- **c.** Transfer 10 µL to the hemacytometer.
- **d.** Count the microspheres within the 4 large corners of the hemacytometer grid.
- **e.** Microspheres/µL = (Sum of microspheres in 4 large corners) x 2.5 x 100 (dilution factor).
- **f.** Note: maximum is 50,000 microspheres/µL.

24. 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: Capture oligo concentration should be optimized for the specific reagents in use. Typically, optimal coupling is achieved using 0.2 to 1 nanomole of capture oligo per 5.0 x 10^6 microspheres as specified in the protocol above.