

## SAMPLE PROTOCOL FOR TWO-STEP CARBODIIMIDE COUPLING OF PROTEIN TO CARBOXYLATED MICROSPHERES

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

1. Resuspend the stock uncoupled microspheres according to the instructions described in the Product Information Sheet provided with your microspheres.
2. Transfer  $5.0 \times 10^6$  of the stock microspheres to a **USA Scientific** microcentrifuge tube.
3. Pellet the stock microspheres by microcentrifugation at  $\geq 8000 \times g$  for 1-2 minutes.
4. Remove the supernatant and resuspend the pelleted microspheres in 100  $\mu\text{L}$   $\text{dH}_2\text{O}$  by vortex and sonication for approximately 20 seconds.
5. Pellet the microspheres by microcentrifugation at  $\geq 8000 \times g$  for 1-2 minutes.
6. Remove the supernatant and resuspend the washed microspheres in 80  $\mu\text{L}$  100 mM Monobasic Sodium Phosphate, pH 6.2 by vortex and sonication for approximately 20 seconds.
7. Add 10  $\mu\text{L}$  of 50 mg/mL Sulfo-NHS (diluted in  $\text{dH}_2\text{O}$ ) to the microspheres and mix gently by vortex.
8. Add 10  $\mu\text{L}$  of 50 mg/mL EDC (diluted in  $\text{dH}_2\text{O}$ ) to the microspheres and mix gently by vortex.
9. Incubate for 20 minutes at room temperature with gentle mixing by vortex at 10 minute intervals.
10. Pellet the activated microspheres by microcentrifugation at  $\geq 8000 \times g$  for 1-2 minutes.
11. Remove the supernatant and resuspend the microspheres in 250  $\mu\text{L}$  of 50 mM MES, pH 5.0 by vortex and sonication for approximately 20 seconds. See **Technical Note 1**.
12. Pellet the microspheres by microcentrifugation at  $\geq 8000 \times g$  for 1-2 minutes.
13. Repeat steps 11. and 12. This is a total of two washes with 50 mM MES, pH 5.0.
14. Remove the supernatant and resuspend the activated and washed microspheres in 100  $\mu\text{L}$  of 50 mM MES, pH 5.0 by vortex and sonication for approximately 20 seconds.
15. Add 125, 25, 5 or 1  $\mu\text{g}$  protein to the resuspended microspheres. (Note: We recommend titration in the 1 to 125  $\mu\text{g}$  range to determine the optimal amount of protein per specific coupling reaction.)
16. Bring total volume to 500  $\mu\text{L}$  with 50 mM MES, pH 5.0.
17. Mix coupling reaction by vortex.
18. Incubate for 2 hours with mixing (by rotation) at room temperature.

19. Pellet the coupled microspheres by microcentrifugation at  $\geq 8000 \times g$  for 1-2 minutes.
20. Remove the supernatant and resuspend the pelleted microspheres in 500  $\mu\text{L}$  of PBS-TBN by vortex and sonication for approximately 20 seconds. See **Technical Note 2**.
21. Incubate for 30 minutes with mixing (by rotation) at room temperature. (Note: Optional – perform this step when using the microspheres the same day.)
22. Pellet the coupled microspheres by microcentrifugation at  $\geq 8000 \times g$  for 1-2 minutes.
23. Remove the supernatant and resuspend the microspheres in 1 mL of PBS-TBN by vortex and sonication for approximately 20 seconds. See **Technical Note 3**.
24. Pellet the microspheres by microcentrifugation at  $\geq 8000 \times g$  for 1-2 minutes.
25. Repeat steps 23. and 24. This is a total of two washes with 1 mL PBS-TBN.
26. Remove the supernatant and resuspend the coupled and washed microspheres in 250-1000  $\mu\text{L}$  of PBS-TBN.
27. Count the microsphere suspension by hemacytometer.

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

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

**Technical Note 1:** 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 2:** 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 3:** Either PBS-TBN or PBS, 0.05% Tween-20 may be used as Wash Buffer.