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Carbodiimide Kit for Carboxylated Microparticles

Introduction:
Polysciences offers the Carbodiimide Kit for covalently coupling proteins to carboxylated microparticles. The contents of the kit are sufficient for at least fifty-five 0.5ml samples (2.5% solids) of carboxylated microparticles. To use the kit for larger samples, increase all volumes in a proportional manner. This procedure is recommended for Microspheres 0.5µ or larger. If using Microspheres smaller than 0.5 microns, please use our Carbodiimide Kit with Hollow Fiber Filtering System (catalog #21758).

Contents of Kit:

Bottle #1	0.1M Carbonate Buffer	200ml
Bottle #2	0.02M Phosphate Buffer	400ml
Bottle #3	Carbodiimide	1g
Bottle #4	0.2M Borate Buffer	450ml
Bottle #5	0.1M Ethanolamine	60ml
Bottle #6	10mg/ml BSA solution	110ml
Bottle #7	Storage Buffer	60ml

Procedure for Coupling Proteins to Carboxylated Microparticles:
NOTE: Centrifuge speed time will vary with particle size.

1. Place 0.5ml of 2.5% suspension of carboxylated microparticles into an Eppendorf centrifuge tube (1.5 - 1.9ml capacity).
2. Add sufficient carbonate buffer (bottle #1) to fill tube and cap tightly.
3. Centrifuge 5-6 minutes or until pelleted in a micro-centrifuge.
4. Carefully remove supernatant using a Pasteur pipette. Discard supernatant.
5. Repeat steps 2, 3, and 4 above a second time.
   To resuspend pellet:
   a) fill tube halfway and cap.
   b) vortex
   c) fill tube to capacity

   NOTE: When term “resuspend pellet” is used, please refer to step 5.
6. Resuspend pellet in phosphate buffer. (bottle #2)
7. Centrifuge 5-6 minutes.
8. Carefully remove supernatant using a Pasteur pipette. Discard supernatant.
9. Repeat steps 6, 7, and 8 above two more times.
10. Resuspend pellet in 0.625 ml of the 0.02M phosphate buffer, pH 4.5
11. Make 0.75ml of a 2% solution of carbodiimide by weighing out 15mg of carbodiimide (bottle #3) and dissolving it in 0.75ml of phosphate buffer (bottle #2).
    NOTE: Carbodiimide solution should be prepared fresh and used within 15 minutes. The carbodiimide in bottle #3 should be protected from moisture. Immediately after weighing out the required amount of carbodiimide, tightly cap the bottle.
12. To the redispersed pellet, add 0.6ml of the 2% carbodiimide solution dropwise and cap the tube tightly. It is best to add dropwise the carbodiimide solution while vortexing.
13. Mix for 3-4 hours at room temperature on a rocker table, rotary shaker, or other type of shaker which provides end-to-end mixing.
14. Centrifuge 5-6 minutes until pelleted and discard supernatant.
15. Resuspend pellet in phosphate buffer (bottle #2).
16. Centrifuge 5-6 minutes or until pelleted and discard supernatant.
17. Repeat steps 15 and 16 two more times. These steps get rid of unreacted carbodiimide.
18. Resuspend pellet in 1ml of borate buffer (bottle #4)
19. Add 200-400µg of protein to be coupled. (We have used rabbit anti-goat IgG, IgG fraction).
20. Leave overnight at room temperature with gentle end-to-end mixing.
21. Centrifuge for 10 minutes. Using a Pasteur pipette, transfer the supernatant completely into a small graduated cylinder or graduated centrifuge tube. Note the volume of the supernatant and save it for protein determination.
    NOTE: If protein determination is done spectrophotometrically, make sure that the supernatant is completely

free of turbidity. This can be achieved by centrifuging the supernatant for an additional 10 minutes. The amount of protein added in Step 19 minus the amount in the supernatant represents the amount bound to the microparticles.

22. Resuspend pellet in 1.2ml of borate buffer. Add 50µl 0.1M ethanolamine (bottle #5) and mix gently for 30 minutes at room temperature. This step serves to block unreacted sites on the microparticles.
23. Centrifuge for 10 minutes and discard supernatant.
24. Resuspend pellet in 1ml of BSA solution (bottle #6) and mix gently for 30 minutes at room temperature. The BSA will block any remaining nonspecific protein binding sites.
25. Centrifuge for 5-6 minutes or until pelleted and discard supernatant.
26. Repeat steps 24 through 25 once, but shorten the mixing time to 5 minutes.
27. Resuspend pellet in 0.5ml to 1ml of storage buffer (bottle #7).

Store the coupled microparticles at 4-6°C. DO NOT FREEZE!

Precautions:

Bottle #6 and #7 contain sodium azide at 0.05% and 0.1% concentration, respectively. Sodium azide is highly toxic. Avoid contact with eyes and skin. Do not pour the contents of bottles #6 and #7 down metal drains. Sodium azide can form explosive mixtures. On disposal, flush with large volumes of water to prevent azide build up.

Carbodiimide (bottle #3) is also toxic. Avoid contact with eyes and skin. Use with adequate ventilation and avoid inhalation.

Ordering Information:

Cat.#	Description	Size
19539	Carbodiimide Kit for Carboxylated Microparticles	1kit

To Order:
In The U.S. Call: 1-800-523-2575 • 215-343-6484
In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767
In Germany FAX: (49) 6221-764620

Order online anytime at **www.polysciences.com**

Related Products:

- Carbodiimide Kit with Hollow Fiber Filtering System (for microspheres 0.1µ - 0.5µ diameter) Catalog #21758
- Carboxylated Paramagnetic Microsphers, 1.2µ Catalog #18598
- Polybead Carboxylated Microspheres sizes ranging from 0.05µ to 10.0µ
- Fluoresbrite Yellow Green (YG 458, 540) Carboxylated microspheres. sizes ranging from 0.05µ to 10µ
- Fluoresbrite Yellow Orange (YO 530, 590) Carboxylated microspheres. sizes ranging from 0.05µ to 6.0µ
- Fluoresbrite Bright Blue (BB 365, 468) Carboxylated microspheres. sizes ranging from 0.05µ to 10.0µ

Please call 1-800-523-2575 for more details on the above Microspheres or for custom bead manufacturing.