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Protocol for Glutaraldehyde Kit

(for use with Amino and Blue Dyed Beads)

Polysciences offers the Glutaraldehyde Kit for covalently coupling proteins to amino functionalized polystyrene beads and blue dyed polystyrene beads. The contents of the kit are sufficient for at least fifty five 0.5ml samples (2.5% solids) of amino beads or blue dyed beads. 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 Glutaraldehyde Kit with Hollow Fiber Filtering System (catalog #23964).

Contents of the Kit

Bottle 1	(Component A)	Phosphate buffered saline (PBS)	3x225ml
Bottle 2	(Component B)	8% Glutaraldehyde in PBS (Labeled Storage Bottle)	empty
Bottle 3	(Component C)	0.2M Ethanolamine in PBS	60ml
Bottle 4	(Component D)	BSA Solution	60ml
Bottle 5	(Component E)	Storage Buffer	60ml
		25% Glutaraldehyde	2 x 10ml

Procedure to prepare 8% Glutaraldehyde in PBS Solution

1. Pipette 10.0ml of Phosphate buffered saline (PBS) into bottle 2.
2. Using ampoule cracker, open 10ml ampoule of 25% Glutaraldehyde.
3. Pipette 5ml of 25% Glutaraldehyde into bottle 2.
4. Mix well, store at 4°C
   NOTE: Glutaraldehyde can be unstable at a pH of 7.4 and may slowly start to polymerize. Please inspect the 8% Glutaraldehyde, PBS solution prior to each use. If turbid or cloudy discard and prepare a fresh solution.

Procedure for Coupling

1. Place 0.5ml of a 2.5% aqueous suspension of beads in an Eppendorf centrifuge tube (1.5 -1.9ml capacity).
2. Add enough PBS (Component A) to fill the tube and cap tightly.
3. Centrifuge for 6 minutes in a microcentrifuge.
4. Remove supernatant carefully using a Pasteur pipette. Discard supernatant.
5. Resuspend pellet in PBS as follows:
   a). Fill tube halfway and cap tightly
   b). Vortex until pellet is completely dispersed
   c). Fill tube close to capacity and cap
   NOTE: When term "resuspend pellet" is used, refer to Step 5 above
6. Centrifuge for 6 minutes and discard supernatant.
7. Repeat steps 5 and 6 once.
8. Resuspend pellet in 0.5ml of 8% glutaraldehyde in PBS (Component B).
9. Mix for 4 to 6 hours at room temperature on a rocker table, rotary shaker, or any other kind of shaker which provides end-to end mixing.
10. Centrifuge for 6 minutes and discard supernatant.
11. Repeat Steps 5 and 6 twice.
12. Resuspend pellet in 1ml of PBS.
13. Add 200-400 micrograms of protein to be coupled.
14. Leave overnight at room temperature with gentle end-to-end mixing.
15. 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 13 minus the amount in the supernatant represents the amount bound to the microparticles.
16. Resuspend pellet in 1ml of 0.2M ethanolamine (Component C) and mix gently for 30 minutes at room temperature. This step serves to block unreacted sites on the microparticles.
17. Centrifuge for 6 minutes and discard supernatant.
18. Resuspend pellet in 1 ml of BSA solution (Component D) and mix gently for 30 minutes and room temperature. The BSA will block any remaining nonspecific protein binding sites.
19. Centrifuge for 6 minutes and discard supernatant.
20. Resuspend pellet in 0.5ml to 1ml of storage buffer (Component E).

Storage
Store the components of kit, coupled microparticles, 25% Glutaraldehyde Ampoules and bottle 2 at 4-6°C. DO NOT FREEZE.

Precautions
Glutaraldehyde is harmful if absorbed through the skin. Avoid contact with eyes, skin or clothing. Avoid breathing vapors. Use only with adequate ventilation. Wear protective gloves and safety goggles. In case of contact immediately flush eyes or skin with plenty of water for at least 15 minutes. Remove contaminate clothing and shoes. Call a physician. Wash clothing and shoes before wearing them again.

Components D and E contain sodium azide at 0.05% and 0.1% concentrations respectively. Sodium azide is highly toxic. Avoid contact with eyes and skin. Do not pour contents down metal drains. Sodium azide can form explosive mixtures. On disposal, flush with large volumes of water to prevent azide build up.

Ordering Information:

Catalog #	
19540	Glutaraldehyde Kit for Amino Beads and Blue Dyed Beads
23964	Glutaraldehyde Kit with Hollow Fiber Filtering System

Other Products:
Polybead Amino Microspheres
Polybead Amino Microspheres are monodisperse latex particles (2.5% solids in water) that contain primary amine surface functional groups. Using glutataldehyde as a coupling agent will result in protein binding 11-12 carbon atoms from the surface of the bead.

Catalog #	Diameter	Size
16586	0.10µ	5ml
15699	0.20µ	5ml
07763	0.50µ	5ml
17144	0.75µ	5ml
17010	1.00µ	5ml
17145	3.00µ	5ml
19118	6.00µ	2ml

Amino Paramagnetic Microspheres

Catalog #	Diameter	Size
18879	1.0µ	2ml
		5ml

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