

Protocol for Glutaraldehyde Kit

(for use with Amino and Blue Dyed Beads)

Introduction

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 0.5 μ m or larger. If using microspheres smaller than 0.5 μ m, please use our Glutaraldehyde Kit with Hollow Fiber Filtering System (Cat. #23964).

Material

Material Supplied

- Bottle 1 (Component A): Phosphate buffered saline (PBS), 3 x 225ml
- Bottle 2 (Component B): 8% Glutaraldehyde in PBS, empty labeled storage bottle
- Bottle 3 (Component C): 0.2 M Ethanolamine in PBS, 60ml
- Bottle 4 (Component D): Bovine Serum Albumin (BSA), 60ml
- Bottle 5 (Component E): Storage Buffer, 60ml
- 25% Glutaraldehyde, 2 x 10ml

Procedure

Researchers are advised to optimize the use of particles in any application, as procedures designed by other manufacturers may not be ideal.

Procedure to Preparing 8% Glutaraldehyde in PBS Solution

1. Pipette 10ml 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 (1.5ml - 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 this step (Step 5).
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 4-6 hours at room temperature on a rocker table, rotary shaker or any other kind of shaker that 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 200-400µg 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 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 0.2 M 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 1ml of BSA solution (Component D) and mix gently for 30 minutes at 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 1.0ml of storage buffer (Component E).

Storage and Stability

Store the components of the kit, coupled microparticles, 25% Glutaraldehyde Ampoules and Bottle 2 at 4°C. Freezing may result in irreversible aggregation and loss of binding activity.

Safety

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 contaminated clothing and shoes. Call a physician. Wash contaminated clothing and shoes before wearing 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 may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Material Safety Data Sheet for more information.

This product is for research use only and is not intended for use in humans or for *in vitro* diagnostic use.

Ordering Information

Catalog Number	Description	Size
19540-1	Glutaraldehyde Kit for Amine and Blue Dyed Microspheres	1 kit
23964-1	Glutaraldehyde Kit with Hollow Fiber Filtering System	1 kit

Related Products

Catalog Number	Description	Size
16586-5	Polybead® Amino Microspheres, 0.10µm	5ml
15699-5	Polybead® Amino Microspheres, 0.20µm	5ml
07763-5	Polybead® Amino Microspheres, 0.50µm	5ml
17144-5	Polybead® Amino Microspheres, 0.75µm	5ml
17010-5	Polybead® Amino Microspheres, 1.00µm	5ml
17145-5	Polybead® Amino Microspheres, 3.00µm	5ml
19118-5	Polybead® Amino Microspheres, 6.00µm	5ml

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Related Products, cont.

Catalog Number	Description	Size
18879-2	Superparamagnetic Amino Microspheres, 1-2 μ m	2ml
18879-5	Superparamagnetic Amino Microspheres, 1-2 μ m	5ml

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