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Glutaraldehyde Coupling Kit

with Hollow Fiber Filtering System

DESCRIPTION

Amine-modified (NH_2) microparticles can be used for covalent coupling of proteins via a homobifunctional amine-reactive crosslinker such as glutaraldehyde (Figure 1).

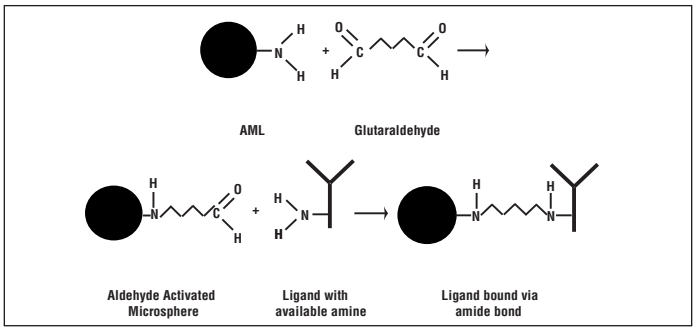

Figure 1: Glutaraldehyde-mediated coupling to amine-modified microspheres.

Polysciences, Inc. offers the Glutaraldehyde Coupling Kit with Hollow Fiber Filtering System for the covalent coupling of proteins to 100-500nm amine-modified microspheres.

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): 0.2M 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 ampoules
- MicroKros hollow fiber filter device

MATERIAL REQUIRED

- Amine-modified polystyrene microspheres
- Microcentrifuge tubes (2ml)
- Rotator (end-over-end)

PROCEDURE

Researchers are advised to optimize incubation times and protein to microsphere ratio for their particular protein. Directions for assembling and operating the MicroKros hollow fiber filter device may be found in Technical Data Sheet #606.

Procedure for Preparing 18% Glutaraldehyde in PBS Solution

1. Pipet 2ml of phosphate buffered saline (PBS) into Bottle 2.
2. Using an ampoule cracker, open a 10ml ampoule of 25% Glutaraldehyde.
3. Pipet 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 bottle of 18% Glutaraldehyde in PBS prior to each use. If turbid or cloudy, discard and prepare a fresh solution.

Procedure for Coupling

1. Pipet 12.5mg of beads into a 2ml microcentrifuge tube (i.e. ~0.5ml of beads at 2.5% solids). Add 1ml of PBS (Component A) and mix by hand-shaking.
2. Transfer the diluted beads to one of the retentate syringes connected to the hollow fiber filter module and concentrate the beads to ~0.75ml (including the 0.5ml hold-up volume) by pushing the retentate syringes back and forth (Figure 2).
3. Detach an empty retentate syringe and add 1ml of PBS to it. Re-attach this retentate syringe to the filtration module.
4. Concentrate the beads again down to 0.75ml (including the 0.5ml hold-up volume).
5. Repeat Steps #3 and 4.
6. Detach the retentate syringe and add 0.75ml of PBS to it. Re-attach this retentate syringe to the filtration module and push the syringe down fully one time.
7. Detach the retentate syringe containing the beads and transfer the washed spheres into a polypropylene tube. Add PBS until the total volume is ~1ml (if necessary). Add 0.75ml of 18% Glutaraldehyde in PBS (Component B).
8. Mix for 4-6 hours at room temperature on a rotator, rocker table, rotary shaker or any other kind of device that provides end-to-end mixing.
9. After incubating, transfer the beads to one of the retentate syringes connected to the hollow fiber filter module and concentrate the beads to ~0.75ml (including the hold-up volume).
10. Detach an empty retentate syringe and add 1ml of PBS to it. Re-attach this retentate syringe to the filtration module.
11. Concentrate the beads again down to 0.75ml (including the hold-up volume).
12. Repeat Steps # 10 and 11, twice.
13. Detach the retentate syringe and add 0.75ml of PBS to it. Re-attach this retentate syringe to the filtration module and push the syringe down fully one time.
14. Detach the retentate syringe containing the beads and transfer the washed spheres into a polypropylene tube. Add PBS until the total volume is ~1.25ml. Mix by inversion or hand-shaking.
15. Re-suspend protein in ~0.5ml of PBS and add to the glutaraldehyde-activated particles. Mix gently end-over-end or briefly vortex. The amount of protein to use during coupling may be determined using Table 1.
16. Incubate protein / bead suspension overnight at room temperature with gentle end-to-end mixing.
17. Transfer the coated beads to one of the retentate syringes connected to the hollow fiber filter module and concentrate the beads to ~0.75ml (including the hold-up volume).

18. Detach the empty syringe and add 0.75ml of PBS to it. Re-attach this retentate syringe to the filtration module and push the syringe down fully one time.
19. Detach the retentate syringe containing the beads and transfer the washed spheres into a polypropylene tube.
20. Save the permeate in the filtrate syringe and transfer it into a small graduated cylinder or graduated centrifuge tube. Note the volume of the permeate and save it for protein determination. *Note:* If protein determination is done spectrophotometrically, make sure that the permeate is completely free of turbidity. The amount of protein added in Step 15 minus the amount in the permeate represents the amount bound to the microspheres.
21. Add 1ml of 0.2M ethanolamine (Component C) to the washed beads. Mix gently for 30 minutes at room temperature. This step serves to block unreacted sites on the microspheres.
22. Transfer the beads to one of the retentate syringes connected to the hollow fiber filter module and concentrate the beads to ~0.75ml (including the hold-up volume).
23. Detach the empty syringe and add 0.75ml of BSA solution to it (Component D). Re-attach this retentate syringe to the filtration module and push the syringe down fully one time.
24. Detach the retentate syringe containing the beads and transfer the washed spheres into a polypropylene tube. Add BSA until the total volume is ~1.25ml. Mix gently for 30 minutes at room temperature. The BSA will block any remaining polymer bead surfaces and minimize nonspecific protein binding in downstream assays.
25. Transfer the blocked beads to one of the retentate syringes connected to the hollow fiber filter module and concentrate the beads to ~0.75ml (including hold-up volume).
26. Detach the empty retentate syringe and add 1ml of Storage Buffer to it (Component E). Re-attach this retentate syringe to the filtration module.
27. Concentrate the beads again down to ~0.75ml (including the hold-up volume).
28. Detach the retentate syringe and add 0.75ml of Storage Buffer to it. Re-attach this retentate syringe to the filtration module and push the syringe down fully one time.
29. Detach the retentate syringe containing the beads and transfer the spheres to a polypropylene tube. Add Storage Buffer until the total volume is ~1ml (if necessary). Mix by inversion or hand-shaking.

Protein Amounts to Use with the Glutaraldehyde Kit Coupling Procedure					
Bead diameter (µm)	0.1	0.2	0.3	0.4	0.5
Protein amount (mg)	2.0 – 5.0	1.0 – 2.5	0.75 – 1.75	0.5 – 1.25	0.4 – 1.0

Table 1

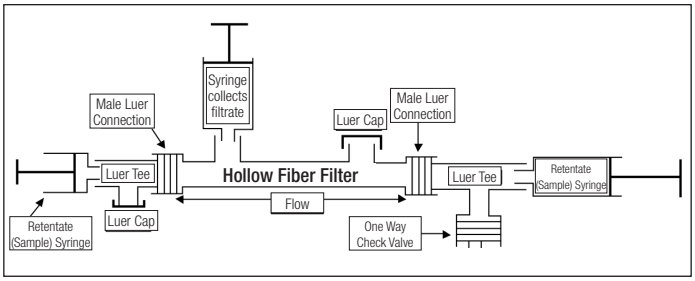

Figure 2: Schematic of MicroKros Hollow Fiber Filter

Note: A single hollow fiber filter module may be used for all of the washes performed for a single coupling reaction. The device may be flushed out between washes to remove residual particles leftover in the hold-up volume.

STORAGE AND SAFETY

Storage

Store the components of the kit, protein-coated microspheres, Bottle 2 and the 25% Glutaraldehyde at 4˚C. Freezing of particles 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, and call a physician. Wash contaminated clothing and shoes before wearing again.

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

ORDERING INFORMATION

Cat. #	Description	Size
23964	Glutaraldehyde Kit with Hollow Fiber Filtering System	1 kit

TO ORDER

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