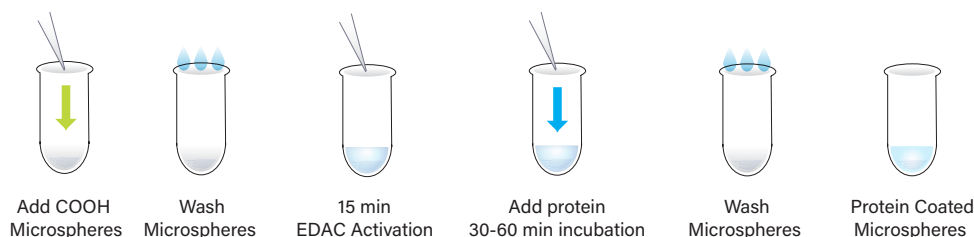


PolyLink Protein Coupling Kit



DESCRIPTION

Bangs Labs PolyLink Protein Coupling Kits provide the necessary reagents for covalent coupling of your protein of interest to polymer, silica, or magnetic microspheres functionalized with carboxyl groups (purchase separately). The -COOH group on the microsphere surface is first activated by EDC or EDAC, a water soluble carbodiimide, to form an active ester. A primary amine group on the protein of interest then reacts with the ester to crosslink the protein to the bead surface.

This kit includes enough reagents for 50 coupling reactions using 200-500 µg of protein per reaction. The procedure outlined below is optimized for coupling carboxyl polymer microspheres and purified IgG. For other proteins or microsphere materials, a series of test ratios and incubation times may need to be performed to ensure the correct amount of protein binds to the surface.

MATERIAL

Material Supplied

- PolyLink Coupling Buffer (50mM MES, pH 5.2; 0.05% Proclin[®] 300): 55mL
- PolyLink Wash/Storage Buffer (10mM Tris, pH 8.0; 0.05% Bovine Serum Albumin; 0.05% Proclin 300): 45mL
- PolyLink EDAC (Carbodiimide): 750mg. Note: Store powder desiccated at -20 °C. Flood headspace with N₂ gas for best preservation. Warm the sealed vial to room temperature in a desiccator to avoid condensate formation in the bottle. Make working solutions just before use.

Material Required

- Carboxylated polymer, silica, or magnetic microspheres
- Centrifuge
- Test tubes

PROCEDURE

Users are advised to optimize the protein to microsphere ratio and incubation times for their particular protein and microspheres.

1. Allow microspheres, PolyLink Coupling Buffer, and PolyLink Wash/Storage Buffer to come to room temperature.
2. Pipet the volume of your microspheres equivalent to 12.5 mg into a 1.5-2 mL polypropylene microcentrifuge tube.
3. Pellet the microspheres via centrifugation for 5-10 minutes at approximately 500-1000 x g. *Note: Centrifugation times will vary according to the size of the particle.*

4. Resuspend microsphere pellet in 0.4 mL of PolyLink Coupling Buffer.
5. Pellet again via centrifugation for 5-10 minutes at approximately 500-1000 x g.
6. Resuspend the microsphere pellet in 0.17 mL of PolyLink Coupling Buffer.
7. Just before use, prepare a 200 mg/mL EDAC solution by dissolving 10 mg PolyLink EDAC in 50 µL PolyLink Coupling Buffer. **Use immediately.**
8. Add 20 µL of the EDAC solution to the microsphere suspension.
9. Mix gently end-over-end or briefly vortex. Allow the activation step to proceed for 15 minutes.
10. Add protein equivalent to 200-500 µg. This may be prepared as 1-5 mg/mL protein in coupling buffer. Mix gently end-over-end or briefly vortex. *Note: The amount of protein bound to the microspheres is dependent on the concentration of protein in solution and on the size of the microspheres. For an example of this relationship, please refer to Figure 1.*
11. Incubate for 30-60 minutes at room temperature with gentle mixing. *Note: End-over-end mixing is best. Longer incubation times may result in greater protein binding. See Figure 2.*
12. Centrifuge mixture for 10 minutes at approximately 500-1000 x g. Save this supernatant for determination of the amount of bound protein.
13. Resuspend microsphere pellet in 0.4 mL PolyLink Wash/Storage Buffer.
14. Repeat Steps 12-13.
15. Store particles at 4-8 °C in PolyLink Wash/Storage Buffer.

NOTES

The PolyLink kit may be used with carboxylated polymer, silica, or magnetic (e.g. ProMag[®], COMPEL™) microspheres. Magnetic separation may be used for superparamagnetic microspheres, or centrifugation steps may be modified for silica, taking its greater density into consideration. The PolyLink protocol may be adapted for spheres that are <1 µm in diameter, e.g. using amount of protein for the increased bead surface area (see TN205), and/or using dialysis or filter devices for separations. Particles in this size range are more prone to aggregation than larger spheres due to their very high surface area:volume ratios, and may require more surfactant and sonication than their larger diameter counterparts. In fact, you may find it useful to sonicate the suspension before, during (e.g. ~every 15 minutes), and after coating. An automated particle sizer can aid in determining the level of monodispersity (i.e. fluctuation in mean diameter), as can traditional microscopy. Although you will not be able to visualize individual 1 µm particles with a standard microscope, aggregates should be visible using 400x magnification.

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Calculation of the Amount of Bound Protein

The amount of protein bound to the microparticles can be determined by subtracting the amount of protein in the supernatants in Steps 12 and 14 from the total starting amount of protein added in Step 10. Protein concentrations of the starting solution and supernatants after binding may be determined by measuring the absorbance at 280 nm or by utilizing commercial protein assay kits. *Note: If measuring absorbance at 280 nm, EDAC may contribute to the absorbance the reading. See TN205 for additional methods to determine the amount of ligand bound.*

Example

$$\frac{(\mu\text{g of protein})_{\text{starting}} - (\mu\text{g of protein})_{\text{washes}}}{\text{mg of microparticles}} = \mu\text{g protein/mL microparticles}$$

$$\frac{(100 \mu\text{g of protein})_{\text{starting}} - (45 \mu\text{g of protein})_{\text{washes}}}{12.5 \text{ mg of microparticles}} = 4.4 \mu\text{g protein/mL microparticles}$$

Expected Results

IgG Binding: Effect of Particle Size and Protein Amount

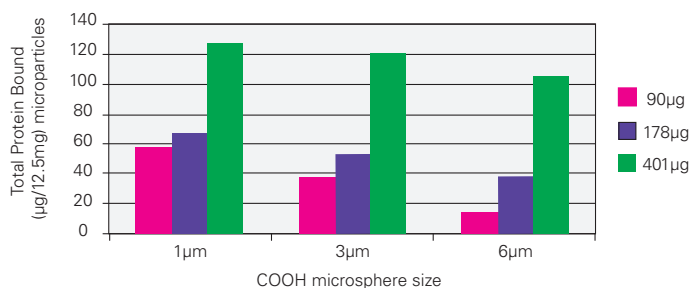


Figure 1: Three sizes of COOH microspheres were exposed to three different levels of Goat anti-Rat IgG protein. The smaller particles represent more surface area per unit of mass and thus bind more total protein.

Time Course of Protein Coupling

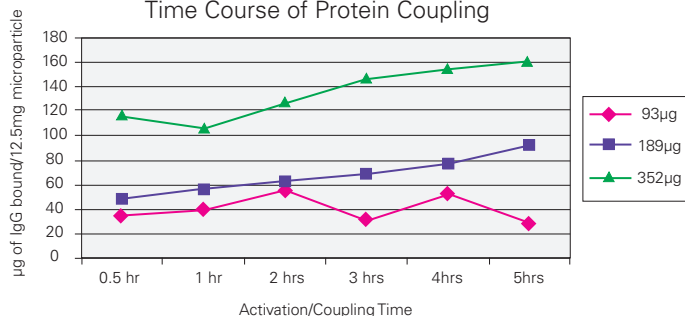


Figure 2: Three different levels of protein were used in the PolyLink procedure with 3µm COOH microspheres. More input protein results in more protein bound. The majority of the protein binding occurs in the first 30 minutes.

STORAGE AND STABILITY

Store PolyLink buffers at 2-8 °C. Coated particles should be stored at 2-8 °C in PolyLink Wash/Storage Buffer or other suitable storage buffer. Store EDAC desiccated at -20 °C. Freezing of particles may result in irreversible aggregation and loss of binding activity.

ADDITIONAL RESOURCES

TechNote 205 Covalent Coupling
 TechNote 203 Washing Microspheres
 TechNote 202 Microsphere Aggregation

ORDERING INFORMATION

Cat. Number	Product Name
PL01N	PolyLink Protein Coupling Kit

Order online at www.bangslabs.com

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