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**B E A D S • A B O V E T H E R E S T™**

## DESCRIPTION

Carboxyl (COOH) microspheres can be used for covalent coupling of proteins by activating the carboxyl groups with water-soluble carbodiimide. The carbodiimide reacts with the carboxyl group to create an active ester that is reactive toward primary amines on the protein of interest.

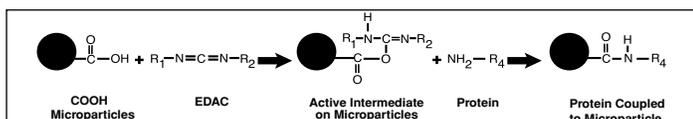


Figure 1

Bangs Laboratories, Inc. offers the PolyLink Protein Coupling Kit for COOH Microspheres for the covalent coupling of proteins to carboxylated microspheres. The procedure that follows has been optimized for polymer microspheres 1µm or larger. The contents of the kit are sufficient for 50 coupling reactions using 200-500µg of protein per reaction. The kit has been optimized using purified IgG as the coupling protein.

## 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 N<sub>2</sub> 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 polystyrene or silica microspheres
- Centrifuge
- Test tubes

## PROCEDURE

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

1. Warm microspheres, PolyLink Coupling Buffer, and PolyLink Wash / Storage Buffer to room temperature.
2. Pipet 12.5mg of microspheres into a 1.5-2mL 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 0.4mL 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 0.17mL of PolyLink Coupling Buffer.
7. Just before use, prepare a 200 mg/mL EDAC solution by dissolving 10mg PolyLink EDAC in 50µL PolyLink Coupling Buffer. **Use immediately.**
8. Add 20µL 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 200-500µg. This may be prepared as 1 - 5mg/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 2.
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 3.
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 0.4mL PolyLink Wash / Storage Buffer.
14. Repeat Steps 12-13, combining supernatants for use in bound protein calculation.
15. Store particles at 2-8°C in PolyLink Wash / Storage Buffer.

## NOTES

### Magnetic Particles

Carboxy-modified superparamagnetic particles are also compatible with the PolyLink kit and magnetic separation may be substituted for centrifugation steps in the procedure when magnetic particles are used.

### Use of PolyLink with Other Microspheres

The PolyLink kit may be used with carboxylated silica or polymer / magnetic (e.g. ProMag™) microspheres. In those instances, magnetic separation may be used for superparamagnetic microspheres, or centrifugation steps may be modified for silica, taking their greater density into consideration.

### Calculation of the Amount of Bound Protein

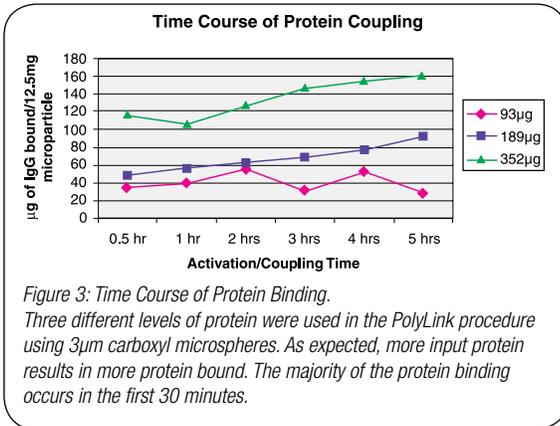
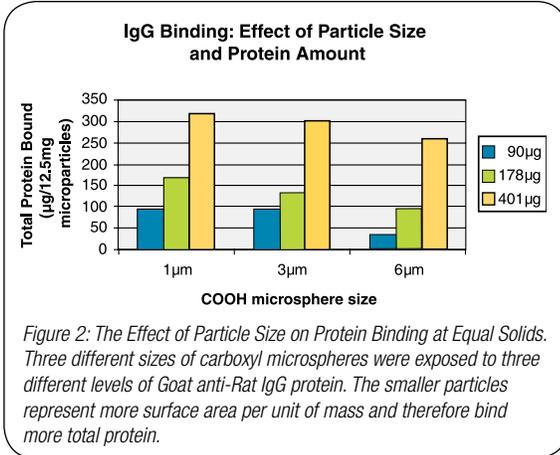
The amount of protein added in Step 10 less the amount of protein left in the supernatants in Steps 12 and 14 represents the amount of protein bound to the microspheres. Protein concentrations of the starting solution and supernatants after binding may be determined by measuring the absorbance at 280nm or by utilizing commercial protein assay kits. *Note:* If measuring absorbance at 280nm, solutions used for calculation of bound protein should not contain EDAC. EDAC may contribute to the absorbance at 280nm.

**Example**

$$\left[ \begin{array}{c} \mu\text{g of protein in} \\ \text{starting solution} \end{array} \right] - \left[ \begin{array}{c} \mu\text{g of protein in} \\ \text{wash supernatants} \end{array} \right] \Bigg/ \begin{array}{c} \text{mg of microparticles} = \\ \mu\text{g of protein bound/mg} \\ \text{microparticles} \end{array}$$

$$\left[ \begin{array}{c} 100\mu\text{g of protein in} \\ \text{starting solution} \end{array} \right] - \left[ \begin{array}{c} 45\mu\text{g of protein in} \\ \text{wash supernatants} \end{array} \right] \Bigg/ \begin{array}{c} 12.5\text{mg of microparticles} = \\ 4.4\mu\text{g of protein bound/mg} \\ \text{microparticles} \end{array}$$

**Expected Results**



**TRADEMARKS AND REGISTERED TRADEMARKS**

1. ProClin® is a registered trademark of Rohm & Haas Company.
2. ProMag™ is a trademark of Polysciences, Inc.

**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.

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

**ORDERING INFORMATION**

Cat. Code	Description	Size
PL01N	PolyLink Protein Coupling Kit	1 kit

Order online anytime at [www.bangslabs.com](http://www.bangslabs.com).