Data Sheet #644

PolyLink Protein Coupling Kit
for COOH Microspheres

9025 Technology Dr. • Fishers, IN 46038-2886
800.387.0672•317.570.7020•Fax317.570.7034
info@bangslabs.com • www.bangslabs.com

Bangs Laboratories, Inc.

B E A D S ● A B O V E T H E R E S T

Carboxyl (COOH) microparticles 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.

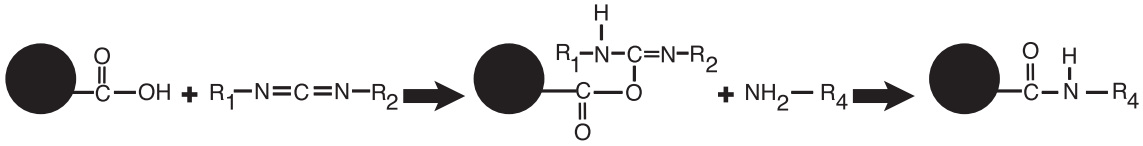

Figure 1

Bangs Laboratories, Inc. is now offering Polysciences' 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 1 micron 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. The user is encouraged to optimize the protein to microsphere ratio and incubation times for their particular protein.

Materials Supplied in the PolyLink Protein Coupling Kit

PolyLink Coupling Buffer (55mL): 50mM MES, pH 5.2, 0.05% Proclin® 300, store at 4°C

PolyLink Wash/Storage Buffer (45mL): 10mM Tris, pH 8.0, 0.05% Bovine Serum Albumin, 0.05% Proclin® 300, store at 4°C

PolyLink EDAC (Carbodiimide) (750mg): **Store powder desiccated @ -20°C.**
Flood headspace with N_2 gas for best preservation.
Make working solutions fresh just before use.

Procedure:

1. Warm microparticles, Coupling Buffer and Wash/Storage reagents to room temperature.
2. Pipet 12.5mg of microparticles into a 1.5 to 2mL polypropylene microcentrifuge tube.
3. Pellet the microspheres via centrifugation for 5 - 10min at approximately 500 - 1000 x G.
   Note: centrifugation times will vary according to the size of the particle.
4. Resuspend microparticle pellet in 0.4mL of PolyLink Coupling Buffer.
5. Pellet again via centrifugation for 5 - 10min at approximately 500 - 1000 x G.
6. Resuspend microparticle pellet in 0.17mL of PolyLink Coupling Buffer.
7. Just before use, prepare a 200mg/mL EDAC solution by dissolving 10.0mg PolyLink EDAC in 50μL PolyLink Coupling Buffer.
   Use immediately.
8. Add 20.0μL of the EDAC solution to the microparticle suspension.
9. Mix gently by end-over-end mixing or by briefly vortexing.
10. Add protein equivalent to 200 - 500μg. Mix gently by end-over-end mixing or by briefly vortexing.
    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 30min to 1 hour 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 10min at approximately 500-1000 x G. Save this supernatant for determination of the amount of bound protein.
13. Resuspend microparticle pellet in 0.4mL PolyLink Wash/Storage Buffer.
14. Repeat steps 12 and 13, combining supernatants for use in bound protein calculation.
15. Store particles at 4°C in PolyLink Wash/Storage Buffer.

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 microparticles. 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.

Example:

[μg of protein in starting solution] − [μg of protein in wash supernatants] / mg of microparticles = μg of protein bound/mg microparticles

[100μg of protein in starting solution] − [45μg of protein in wash supernatants] / 12.5mg of microparticles = 4.4μg of protein bound/mg microparticles

***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.

Expected Results:

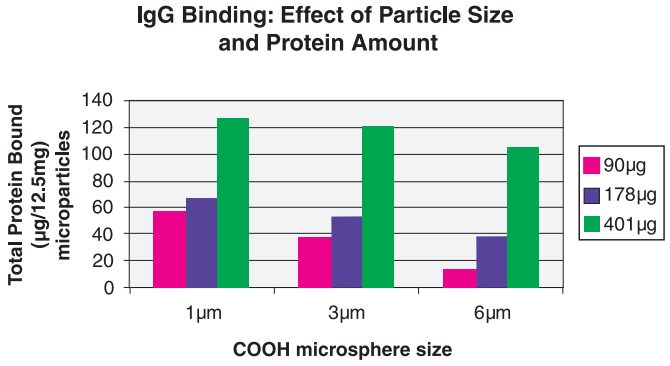

Figure 2: The Effect of Particle Size on Protein Binding at Equal Solids. Three different sizes of carboxyl microspheres were exposed to three different levels of Goat anti-Rat IgG protein. The smaller particles present more surface area per unit of mass and therefore bind more total protein.

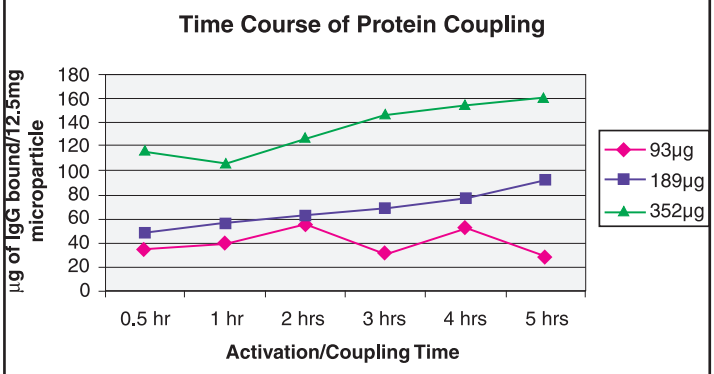

Figure 3: Time Course of Protein Binding. Three different levels of protein were used in the PolyLink procedure using three micron carboxyl microspheres. As expected, more input protein results in more protein bound. The majority of the protein binding occurs in the first 30 minutes.

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

Ordering Information:	**Catalog Code**	**Description**	**Size**
	PL01N	PolyLink Protein Coupling Kit	1 kit

Order online anytime at www.bangslabs.com or call Customer Service at 317.570.7020 or 800.387.0672 US