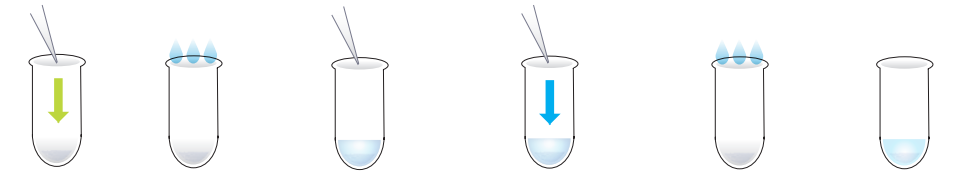
Bangs Laboratories, Inc.
BEADS ABOVE THE REST

PolyLink Protein Coupling Kit

Product Data Sheet 644



DESCRIPTION

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.

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_2 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

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

1. Warm microparticles, PolyLink Coupling Buffer, and PolyLink Wash / Storage Buffer to room temperature.
2. Pipet 12.5mg of microparticles into a 1.5-2mL polypropylene microcentrifuge tube (or Vivaspin® device).
3. Pellet the microparticles 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 microparticle 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 microparticle 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 microparticle 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 microparticles is dependent on the concentration of protein in solution and on the size of the microparticles.* 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 microparticle pellet in 0.4mL 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 <1µm in diameter, e.g. using amount of protein for the increased bead surface area (see TN205), and/or using our Vivaspin® Ultrafiltration devices (cat # AA022) 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 1µm particles with a standard microscope, aggregates should be visible using 400x magnifcation.

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. *Note: If measuring absorbance at 280nm, EDAC may contribute to the absorbance the reading.* See TN205 for additional methods to determine the amount of ligand bound.

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

Expected Results

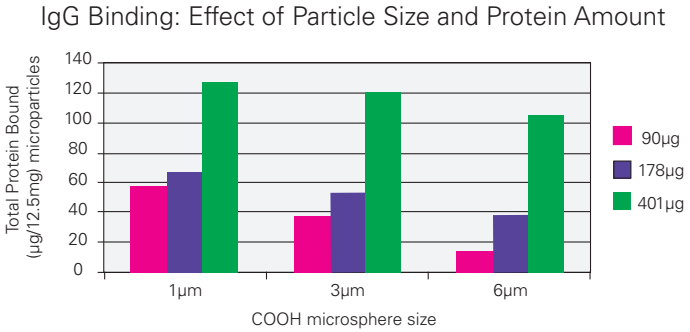


Figure 1: *Three sizes of COOH microspheres were exposed to three different levels of Goat anti-Rat IgG protein. The smaller particles represent more surface area per unit of mass and thus bind more total protein.*

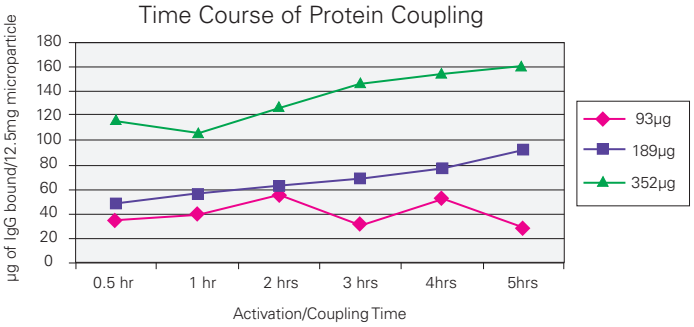


Figure 2: *Three different levels of protein were used in the PolyLink procedure with 3µm COOH microspheres. More input protein results in more protein bound. The majority of the protein binding occurs in the first 30 minutes.*

TRADEMARKS

ProClin® is a registered trademark of Rohm & Haas Company.
ProMag® is a registered trademark of Polysciences, Inc.
Vivaspin® is a registered trademark of Sartorius Stedim Biotech GmbH, Goettingen, Germany.

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

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/Units
PL01N	PolyLink Protein Coupling Kit	1 kit
AA022	Vivaspin® 2mL Ultrafiltration Device (for beads <0.5µm)	5 Units

Order online anytime at **www.bangslabs.com.**