

## **Derivatization of Polymer Microspheres**

Polystyrene - polyethylene glycol (PS-PEG) graft copolymer microspheres ( $\approx 130 \mu\text{m}$  in diameter when dry and  $230 \mu\text{m}$  when hydrated) were purchased from Novabiochem. Normal amine activation substitution levels for these beads were between 0.2 and 0.4 mmol/g. Commercial-grade reagents were purchased from Aldrich and used without further purification except as indicated below. Fluorescein isothiocyanate was purchased from Molecular Probes. All solvents were purchased from EM Science and those used for solid-phase synthesis were dried over molecular sieves. Methanol was distilled from magnesium turnings.

Immunoassays were performed using carbonyl diimidazole (CDI) activated Trisacryl® GF-2000 available from Pierce Chemical (Rockford, IL). The particle size for this support ranged between 40 and  $80 \mu\text{m}$ . The reported CDI activation level was  $> 50 \mu\text{moles/mL}$  gel. Viral antigen and monoclonal antibody reagents were purchased from Biodesign International (Kennebunk, ME). Rhodamine and Cy2 -conjugated goat anti-mouse antibody was purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Antigen and antibody reagents were aliquoted and stored at  $2-8^\circ \text{C}$  for short term and at  $-20^\circ \text{C}$  for long term. Goat anti-mouse antibody was diluted with glycerol (50%) / water (50%) and stored at  $-20^\circ \text{C}$ .

Agarose beads (6% crosslinked) used for the enzyme-based studies were purchased from XC Bead Corp. (Lowell, MA). The beads were glyoxal activated ( $20 \mu\text{moles}$  of activation sites per milliliter) and were stored in sodium azide solution. Agarose bead sizes ranged from  $250 \mu\text{m}$  to  $350 \mu\text{m}$ .

## **General Procedures**

All final functionalized PS-PEG copolymer microsphere batches (resin) were dried under high vacuum for at least twelve hours. The resin was washed thoroughly before and after each coupling reaction on the solid phase using a rotary evaporator motor to tumble the reaction vessel in an oblong fashion (shaking), for a specified period of time (i.e., the "1 x 1" notation refers to one wash for one minute before the solvent was drained).

**Solid phase peptide synthesis forming an amide linkage between the indicator and the resin (i.e. alizarin complexone and o-cresolphthalein complexone)** Amino-terminated polystyrene - polyethylene glycol graft copolymer resin (0.20 g, 0.29 mmol/g, 0.058 mmol) was placed in a solid phase reaction vessel and washed with 1 x 1 minute dichloromethane, 2 x 5 minutes N,N-dimethyl formamide (DMF), and 2 x 2 minutes dichloromethane. While the resin was being washed, an oven-dried round-bottom flask was charged with dicyclohexylcarbodiimide (DCC) (0.059 g, 0.29 mmol, 5 eq.) and hydroxybenzotriazole (HOBt) (0.039 g, 0.29 mmol, 5 eq.) in 8 mL DMF and cooled in an ice-bath. To this mixture, alizarin complexone (0.20 g, 0.29 mmol, 5 eq.) was added and the solution stirred at 0 °C for 30 minutes. After completing the washes of the resin, this solution was filtered and added to the resin. The heterogeneous system was allowed to shake for 2-15 hours at 25 °C. At the end of this time, the coupling solution was removed and the resin was washed with 2 x 2 minute DMF, 1 x 2 minute dichloromethane, 1 x 2 minute methanol, 1 x 5 minute DMF and 1 x 1 minute dichloromethane. A small portion of this resin was then subjected to a quantitative ninhydrin (Kaiser) test to assay for the presence of primary amines, using Merrifield's quantitative procedures. Various indicator substitution levels were used as required for the desired assays.

Other dyes such as xylenol orange (Sigma), calconcarboxylic acid (Aldrich) and thymolphthalexon (Aldrich) were conjugated to the resin beads using similar protocols as described above.

**Acetylated resin.** Prewashed resin (0.10 g, 0.29 mmol/g, 0.029 mmol) was treated with acetic anhydride (1.5 mL, 15.9 mmol, 548 eq.) and triethylamine (0.034 g, 7.2 mmol, 248 eq.) in 5 mL dichloromethane. After 30 minutes of shaking at 25 °C, the reaction mixture was removed and the resin was washed (as described above). A ninhydrin test produced a negative result.

**Solid phase peptide synthesis forming a thiourea linkage between the indicator and the resin (fluorescein).** Once the resin (0.075 g, 0.30 mmol/g, 0.0218 mmol) had been completely washed, fluorescein isothiocyanate (0.034 g, 0.087 mmol, 4 eq.) in 5 mL dichloromethane and 5 mL DMF was added to it. Two

different levels of dye loading were created so as to service the specific needs of the colorimetric and fluorescence-based measurements. If the resin was to be used for colorimetric studies, it was allowed to shake in an oven at 55 °C for 1-5 days. The subsequent work-up of washes was followed as previously mentioned. If a positive ninhydrin test was obtained, the resin was resubmitted to the reaction conditions until ninhydrin gave a negative result. Resin designated for fluorescence studies was shaken at 25 °C only for 1-3 days as lower dye loading was needed. A quantitative ninhydrin test was then performed to assess the level of substitution. A low loading volume was required to minimize fluorescence self-quenching.

***Viral Immunoassays.*** Hepatitis B surface antigen (HbsAg) was coupled to the CDI-activated Trisacryl support in the following manner: 20  $\mu$ L of a 50% (by volume) bead slurry was pipetted into a 0.6 mL microcentrifuge tube. The number of moles activated CDI sites per mL bead slurry was determined and reacted with HBsAg in a 1:3000 ratio (1 mole protein: 3000 moles CDI sites). To the microcentrifuge tube was added 500  $\mu$ L of a solution of phosphate buffered saline at pH 8. The resulting reaction mixture was allowed to react overnight at RT with shaking. Similar procedures were performed with HIV gp 41/120 and influenza A antigens.