Carboxylated Beads-Protein G-Antibody Coupling

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Materials

activation buffer
100 mM MES (Sigma M-8250)
0.5 M NaCl
Adjust the pH to 6.0 with NaOH
1% Tween20 (Sigma P-7949)

100mM PBS; pH 7.0 (50 ml)
140 mM (1.4ml 5M) NaCl
2.7 mM (67.5λ 2M) KCl
61 mM (522mg) K_2HPO_4
39 mM (272mg) KH_2PO_4
Adjust the pH to 7.0 with HCl

100mM PBS; pH 7.4 (50 ml)
140 mM (1.4ml 5M) NaCl
2.7 mM (67.5λ 2M) KCl
80 mM (696mg) K_2HPO_4
20 mM (136mg) KH_2PO_4
Adjust the pH to 7.4 with HCl

ab X-linking
100 mM Na_2HPO_4 pH 8.5
100 mM NaCl (or other non-amine containing buffer pH 7-9).

A. Spherotech polystyrene carboxylated beads, 5% w/v.
B. Protein G (Pierce 5 mg 21193) Dissolve in 1 ml PBS pH 7.4.
C. Sulfo-NHS (Pierce 24510)
D. EDC (Pierce 22980)
E. 2-mercaptoethanol (Sigma M3148)
F. Crosslinker: DMP (add 50 mg to 1 ml of crosslinking buffer, dissolve, and immediately add 30 μl to 1 ml reaction) The desired final concentration is 10 mM. NOTE: you can also buy DMP in 50 mg vials.
G. Sheep polyclonal anti-dig antibody (Roche 1333 089). Dissolve in 200 μl PBS pH 7.4. (1 mg/ml antibody)

Method

1. Spin 1 ml beads at 3000 rpm for 3 min. Resuspend beads in 1 ml activation buffer.

2. Dissolve 10 mg EDC and 22 mg sulfo-NHS in 1 ml deionized water, vortex to dissolve the chemicals and immediately add 100 μl to the beads. This corresponds to about ~5 mM EDC and ~10 mM sulfo-NHS. The molarities are doubled with respect to the standard Pierce protocol. React for 15 min at room temperature.

3. Add 2.8 μl of 2-mercaptoethanol (final concentration 40 mM) to quench the reaction. Wait ten minutes at RT. Spin down and resuspend in 1 ml MES activation buffer (3x).

4. Add 1 mg of protein G (200 μl). Allow the protein and the beads to tumble for 3 hours at room temperature.

5. Spin down and wash with 1 ml PBS 7.4. Spin down and resuspend beads/protein G in 1 ml PBS 7.0 (for storage) OR in 1 ml of Ab X-linking buffer.

6. Add 60 μl of the Roche polyclonal antibody and 30 μl of dissolved DMP. Tumble at RT for 60 min. Spin down and resuspend in 1 mL 2 M Tris (base) and wait for two hours in vortex at minimal speed (this is to quench the reaction). Then dilute the beads 3 fold with PBS, pH 7.0, spin down and resuspend in PBS, pH 7.0. (The three fold dilution with PBS pH 7.0 is necessary because 2 M Tris is too dense to allow the beads to precipitate). I span down and resuspended the beads in PBS pH 7.0 three times. The final product should be kept at neutral to acidic pH since imidoamides slowly hydrolyze at high pH's.

Notes:

Imidoester reactions are carried out at 0°C or room temperature as it has been shown that elevated temperatures are principle causes of poor yields with these reactions.

The DMP reaction can be terminated with Glacial Acetic Acid at a 1:4. Alternatively, an amine containing solution like Tris or glycine can be added to quench the reaction.

1. Store the crosslinkers desiccated at temperature indicated on the vial label.

2. Allow the vial to warm completely to room temperature before opening.

3. Unopened, these crosslinkers are stable for at least one year. After opening, they should be stored protected from moisture and used within 6 months.

4. Phosphate, carbonate, Hepes, Triethylamine, N-ethyl morpholineacetic acid buffers, free of primary amines, between pH 7-9 (8-9 optimal), may be used for the DMP step.

5. A 10-30 fold molar excess of DMP crosslinker over primary amine containing molecules is recommended.

6. When crosslinking two different proteins, mix the proteins in a 1:1 ratio then add the crosslinker.

7. If a stock solution of the crosslinker is used it should be prepared immediately before use.

8. Once the protein G is dissolved in buffer, it can be stored for two months at 4 degrees, or for years at –20 degrees (after having added 50% glycerol).