Micro-oxen protocols:

ConA + beads

Adsorbing ConA to polystyrene beads

by Saber Khan, Jason Park
(Last updated:)

Reagents list

Conjugation buffer
    pH 7.0 PBS (See notes on pH below; the pI of ConA is 4.5-5.5)
ConA
    (Note: ConA-biotin version is ok unless biotin will be used in other downstream protocols)
    (Sigma C2272)
    Lyophilized; Resuspended in ddH2O @ 10 mg/mL
    (Sidenote: ConA must bind to a transition metal and to Ca2+ for binding activity)
    (Note: Succinyl-ConA is dimer-only, has different biological activity, may be useful to try)

Step-by-step

1) 114.6 uL (1 mg) of 1um diameter YG FluoresBrite beads (stock concentration (?/??/??): 1.82% solids)
2) Add 185.7 uL of conjugation buffer to get 1% solids beads (pH 7.0 PBS)
3) 4.62 uL of 10 mg/mL ConA-biotin (46.2 ug - see notes on ratio below) in a tube. Add contents of step 1.
4) Shake gently 1-2 hrs @ room temperature.
5) Wash:
    Centrifuge at 10,000 g for 5 mins
    Resuspend in 1 mL storage buffer (final concentration ~1 mg/mL)
    (Do 3 times)

Notes, hints, rationale behind protocol decisions

Deciding on molar ratio of ConA to beads

3-10x excess of protein over calculated monolayer concentration is standard for protein adsorption to polystyrene beads (ref Bioconjugate Techniques p592). (Protocol reference: Bangs TechNotes 204: Adsorption to Microspheres).

MONOLAYER CALCULATION:
$S = (6 / \rho D)(C)$

WHERE:
S = amount of representative protein needed to achieve surface saturation (mg protein/g of microspheres),
C = capacity of microsphere surface for given protein, which will vary depending on the size and molecular weight of the protein to be coupled (mg protein/m2 of polymer surface),
6 / ρD = surface area/mass (m2/g) for microspheres of a given diameter (ρ = density of microspheres, which for polystyrene is 1.05 g/cm3),
D = diameter of microspheres, in microns.

Examples:
For BSA (MW 65kD), C ~ 3 mg/m2
For bovine IgG (BIgG, MW 150kD), C ~ 2.5 mg/m2
For BIgG: C ~ 2.5 mg/m2, so:
S = (6 / ρD)(C)
= (6 / 1.05 g/cm3 • 1.0μm)(2.5 mg/m2)
~ 15 mg of BIgG to saturate 1 gram of 1μm polystyrene-based microspheres.

MW of ConA:
26.5kDa per monomer unit (mostly dimer at pH 4.5-5.6, but turns tetramer at pH > 7.0)

S = (6 / 1.05 g/cm3 * 1.0um)(2.75 mg/m2)
S = **15.7 mg of ConA calculated monolayer for each 1 gram of 1um polystyrene microspheres.**

pH choice

It is generally best to adsorb the protein at a pH close to the pI of the protein being adsorbed. This is because protein adsorption to hydrophobic microspheres happens as a result of hydrophobic and van der Waals interactions.

The pI of ConA is 4.5-5.5.

Other

Note that passive adsorption of proteins usually results in a very high percentage (most!) of the protein getting misfolded / denatured (based on antibody adsorption studies).

Coupling streptavidin beads to biotin-ConA by streptavidin-biotin bond

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(Last updated:)

Reagents list

Washing/Blocking buffer

PBS + x% SuperBlock in PBS
Storage buffer
ConA-biotin (Sigma C2272)

Step-by-step

1) WASH / BLOCK STEP: Wash (1 mg polystyrene; 2 mg silica (200 uL of stock); 2 mg Dynabeads (200 uL of stock)) of streptavidin-beads in 1mL of Washing/Blocking buffer.
2) Spin down, 10,000g x 5 min
(What is the best speed? Depends on size of particle, tendency to aggregate incl hydrophobicity / hydrophilicity) (Silica-trial 1 - 2000g/5min)
For Dynabeads, use magnetic accessory instead of centrifugation.
3) Repeat 1 and 2 three times.
4) BINDING STEP: Incubate beads w/ conA-biotin (1.54 uL of 10mg/mL) (15.4 ug ConA-biotin per 1 mg of PS beads or 2mg of silica beads) for 30 min @ 4C.
4) WASH STEP: Spin down and resuspend in (1 mL) Storage buffer, 3x.
For Dynabeads, use magnetic accessory instead of centrifugation.
5) Store in 4 C refrigerator until ready for use.