Luminex Bead Coupling

Aaron Meyer

Abstract

We use this process to create our own Luminex assays from standard ELISA reagents.

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Guidelines

Minimize the exposure of EDC and S-NHS to air and moisture. Use fresh aliquots for each coupling reaction and discard after use. Pierce sells individually packaged, small amounts of S-NHS that can be used in a single use fashion.

Before start

Activation buffer (100 mM NaH2PO4, pH 6.3)
Coupling buffer (50 mM HEPES, pH 7.4)
PBS
PBS/1% BSA
EDC (N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide)
S-NHS (N-hydroxysulfosuccinimide)

Protocol

Step 1.
Vortex the bead stock suspension to yield a homogeneous bead suspension.

Step 2.
Dissolve approximately 10 mg each of EDC and S-NHS into 2 microcentrifuge tubes and resuspend in deionized water at 50 mg/mL.

Step 3.
Centrifuge the 100 µl bead suspension at 10,000 x g.

DURATION
00:03:00

Step 4.
Carefully remove and discard the supernatant.

Step 5.
Resuspend the beads in 80 µl activation buffer.

Step 6.

Add 10 µl of S-NHS solution and 10 µl of EDC solution to the bead suspension.

Step 7.
Incubate with agitation at room temperature in the dark at roughly 900 rpm.

DURATION
00:20:00

Step 8.
Dilute your protein stock solution with coupling buffer to a concentration of 0.1 mg/ml in a volume of 100 µl. Optimal coupling may occur at a concentration within 25-250 µg/ml. Note the protein stock cannot have any other amine groups present.

Step 9.
Centrifuge the beads at 10,000 x g.

DURATION
00:03:00

Step 10.
Carefully remove and discard the supernatant.

Step 11.
Add the diluted protein solution.

Step 12.
Agitate the tube with activated beads and protein solution overnight at 4C at roughly 900 rpm in the dark (wrapped in foil).

Step 13.
Centrifuge the beads at 10,000 x g. Discard supernatant.

DURATION
00:03:00

Step 14.
Wash the beads three times with PBS/1% BSA.

Step 15.
Resuspend the bead pellet in 1 ml PBS/1% BSA.

Step 16.
Determine bead concentration using Luminex and adjust amount of stock used accordingly.