

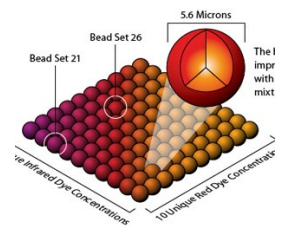
Jun 18, 2019

Version 1

🌐 Phagocytosis Bead Conjugation V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.4cxgsxn>



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Protocol Citation: Andrew Crowley 2019. Phagocytosis Bead Conjugation. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.4cxgsxn>



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Protocol status: Working

We use this protocol and it's working

Created: June 18, 2019

Last Modified: June 18, 2019

Protocol Integer ID: 24695

Keywords: microsphere, bead, phagocytosis, Luminex, fluorescent, phagocytosi, amine coupling

Abstract

Protocols for the amine coupling of microspheres

Materials

STEP MATERIALS

 PBS-TBN Teknova Catalog #P0210

 PBS-TBN Teknova Catalog #P0210

Protocol materials

 PBS-TBN Teknova Catalog #P0210

 PBS-TBN Teknova Catalog #P0210

Activation

- 1 Calculate the beads / mL from the percent solids as follows:

$$(6W \times 10^{12}) / (\rho \times \pi \times \varphi^3)$$

W = grams solid per mL

ρ = density (g/mL)

φ = particle diameter (μm)

polystyrene density = 1.055 /mL

- 2 Resuspend beads by briefly vortexing; dispense

 200 μL (contains approx. 7 billion beads)

- 3 Pellet by centrifugation  17000 x g  00:03:00 and remove supernatant by pipette

- 4 Resuspend in a total of  1 mL consisting of:

- sulfo-NHS  50 mg/mL made in  10 millimolar (mM) MES (pH 5.0)

 100 μL

- EDC  50 mg/mL , made in  10 millimolar (mM) MES (pH 5.0)  100 μL

-  10 millimolar (mM) MES (pH 5.0)  800 μL



- 5 Incubate with end-over-end mixing (shielded from light) ⌚ 00:20:00

🌡 Room temperature

Coupling

- 6 Wash by pelleting beads and replacing supernatant with [M] 10 millimolar (mM) MES

(pH 5.0) 🧪 1 mL

🌀 17000 x g ⌚ 00:02:00

- 7 Formulate protein for coupling @ approx. [M] 25 µg/mL in [M] 10 millimolar (mM)

sodium acetate (pH 5.0) 🧪 7.5 mL

Note

n.b.

The large volume used for the step is based on observations that sufficiently high bead concentrations during this step may lead to crosslinking between microspheres (**anecdotal**)

- 8 Incubate with end-over-end mixing (shielded from light) ⌚ 03:00:00

🌡 Room temperature

Blocking + Washes

- 9 Collect the full volume in the base of the reaction tube by centrifugation 🌀 250 x g

⌚ 00:01:00



- 10 Split the  7.5 mL volume into multiple fractions in microcentrifuge tubes ( 2 mL) in order to centrifuge at a sufficiently high speed  17000 x g  00:03:00
- 11 Remove supernatant from each fraction by pipette
- 12 Resuspend **one** fraction in PBSF (ie. 1x PBS +  0.1 Mass / % volume BSA)  1 mL
- 13 Transfer the entire contents of the first fraction to the second and resuspend; continue through the fractions until as many beads as possible have been collected in a single microcentrifuge tube
- 14 Pellet beads via centrifuge  17000 x g  00:02:00
- 15  [go to step #11](#) for a total of 3 washes
- 16 Resuspend in PBSF  500 µL
- 17 Will the beads be used for an assay on the same day as production?

STEP CASE

No  16 steps

- 18 Block via  16:00:00 (ie. overnight)  4 °C

Counting + Qualification

- 19 Make serial dilutions of 1:100, 1:1000, and 1:10,000



- 20 Measure concentration by flow cytometry using the fluorescent dye of the microsphere as the trigger condition

Expected result

Typical yield is approx. $1 - 2 \times 10^8$ beads, or around 25% of the original amount

Equipment

MACS Quant	NAME
flow cytometer	TYPE
Miltenyi	BRAND
130-096-343	SKU

- 21 In a non-binding, 96-well plate, formulate serial dilutions of positive and negative control antibodies beginning at **5 μ g/mL** and diluted by 1:3 over an approx. 4 point series
- 22 Add 100,000 beads to each well
- 23 Incubate with shaking (shielded from light) **01:00:00** **Room temperature**
- 24 Pellet by centrifugation **3500 x g** **00:05:00** and remove supernatant by decanting



25 Resuspend beads in PBST (ie. 1x PBS + [M] 0.05 % volume Tween20)  200 μ L

26  go to step #24 for a total of 2 washes

27 Resuspend beads in fluorescent secondary  100 μ L [M] 0.65 μ g/mL

Note

n.b.

Make sure that the fluorescent molecule chosen for the secondary does not have spectral overlap with the dye in the microspheres themselves

28 Incubate with shaking (shielded from light)  00:30:00  Room temperature

29 Pellet by centrifugation  3500 x g  00:05:00 and remove supernatant by decanting

30 Resuspend beads in PBST (ie. 1x PBS + [M] 0.05 % volume Tween20)  200 μ L

31 Pellet by centrifugation  3500 x g  00:05:00 and remove supernatant by decanting

32 Resuspend beads in 1x PBS  100 μ L

33 Measure fluorescent intensity by flow cytometry

Equipment

MACS Quant

NAME

flow cytometer

TYPE

Miltenyi

BRAND

130-096-343

SKU