VERSION 2

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Protocol status: Working
 We use this protocol and it's working

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🌐 Phagocytosis Bead Conjugation V.2

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ABSTRACT

Protocols for the amine coupling of fluorescent microspheres with antigen or capture reagent for opsonization and phagocytosis

Quantity as written: approx. 1×10^8 under typical yields (enough for 400 phagocytosis wells under standard protocol)

MATERIALS

STEP MATERIALS

⊗ PBS-TBN Teknova Catalog #P0210

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Activation

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

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

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 **10 millimolar (mM)** MES (pH 5.0)

1 mL

17000 x g 00:02:00

- 7 Formulate protein for coupling @ approx. **25 µg/mL** in **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 + [M] 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 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 17 includes a Step case.
No
Yes

step case

No

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 µg/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 + 0.05 % volume Tween20) 200 μ L
- 26 for a total of 2 washes
- 27 Resuspend beads in fluorescent secondary 100 μ L 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 + **1mM 0.05 % volume** Tween20) **200 µL**
- 31 Pellet by centrifugation **3500 x g** **00:05:00** and remove supernatant by decanting
- 32 Resuspend beads in 1x PBS **100 µL**
- 33 Measure fluorescent intensity by flow cytometry

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