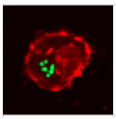

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Phagocytosis Bead Conjugation V.2

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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 x 10^8 under typical yields (enough for 400 phagocytosis wells under standard protocol)

STEPS MATERIALS

NAME	CATALOG #	VENDOR
PBS-TBN	P0210	Teknova
PBS-TBN	P0210	Teknova

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 appox. 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 Milimolar (mM)** MES (pH 5.0) **100 µl**
- EDC **50 mg/mL** , made in **10 Milimolar (mM)** MES (pH 5.0) **100 µl**
- **10 Milimolar (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 Milimolar (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 Milimolar (mM) sodium acetate (pH 5.0) 7.5 ml

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 Percent** 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

no description provided

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

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

MACS Quant
flow cytometer
Miltenyi 130-096-343

21 In a non-binding, 96-well plate, formulate serial dilutions of positive and negative control antibodies beginning at [M]**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 + [M]0.05 Volume Percent Tween20) 200 µl

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

27 Resuspend beads in fluorescent secondary 100 µl [M]0.65 µg/mL

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 Percent 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

MACS Quant
flow cytometer
Miltenyi 130-096-343

Washing + Blocking

step case

No

no description provided

NaN Block via **16:00:00 (ie. overnight)** **4 °C**

Counting + Qualification

NaN

Washing + Blocking

step case

Yes

no description provided

NaN Block via **00:30:00** **Room temperature**

Blocking + Washes

step case

Yes

no description provided

18 Block via **00:30:00** **Room temperature**

Counting + Qualification

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