MANNOSYLATED INTRAPHAGOSOMAL ASSAY PARTICLE PREPARATION

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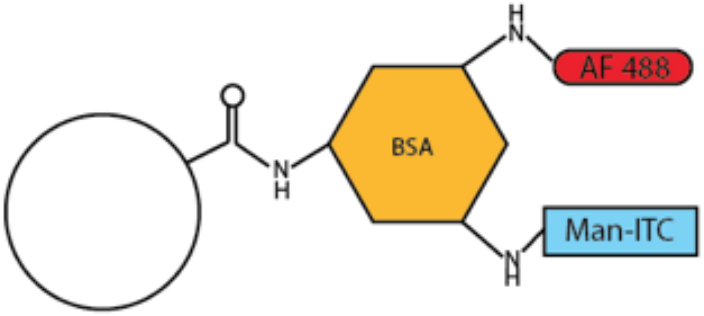
BACKGROUND

Particle preparation for spectrofluorometric and microscopy based assays of intraphagosomal phagolysosomal fusion.



MATERIALS

- 3µM carboxylated microspheres suspension (Kisker Biotech)
- PBS
- Cyanamide
- Coupling buffer (0.1M Borate buffer pH 8.0)
- Defatted BSA
- AF 488-SE
- AF 594- SE
- Man-ITC
- 3M Borate buffer pH 9.0

PROTOCOL

PART A: Making Man-BSA Bead

*use static-free polypropylene tubes

1. Take 1mL of 3µm carboxylated microspheres suspension (50mg) spin down at 500x *g* for 30 seconds, remove supernatant and wash with PBS. Repeat wash step.
2. Spin down, remove supernatant and add 50mg of carbodiimide/cyanamide dissolved in 1mL PBS. Vortex and nutate for 15 minutes at room temperature (RT).
3. Spin down, remove supernatant and wash 3 times with coupling buffer.
4. Spin down, remove supernatant and add 20mg defatted BSA in 1mL coupling buffer. Nutate for 2 hours in RT.
5. Wash twice with PBS and continue on to Part B (Mannosylated beads) or Part C (Maleylated beads).

PART B: Labeling

1. Wash beads twice in coupling buffer. Resuspend in 500µL coupling buffer.
2. Add 10 uL of 5mg/mL stock AF488 SE and 10 uL of 2.5 mg/mL man-ITC (dissolved in DMSO) nutate for 30 minutes in the dark at RT
3. Wash the beads twice in PBS and store at 4 °C.