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Extracellular vesicle isolation from bacterial cultures

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1 Works for me dx.doi.org/10.17504/protocols.io.8fzhtp6

Extracellular Vesicles

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ABSTRACT

Steps for isolating extracellular vesicles and other small particles from a bacterial culture

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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Culturing

1 Grow bacterial culture to mid/late exponential phase

Vesicle+Particle Isolation

2 Remove cells by filtration through a 0.2µm filter and collect the filtrate (<0.2µm fraction). Depending on the culture, the bulk cell mass can be first removed by gentle centrifugation (~10,000 xg or less) prior to filtration.

Whatman Polycap TC 0.2 µm capsule filter
capsule filter
Whatman 6717-9502

3 Concentrate <0.2µm, cell-free supernatant using a tangential flow filter (100 kDa cutoff). Try to keep feed pressure <10 psi. Try to get the final volume as low as possible.

4 Re-filter concentrated material through a 0.2 µm syringe filter. Pellet the vesicles in an ultracentrifuge at 100,000 xg, for at least 1 hr, at 10 C or lower.

5 A pellet will not necessarily be visible. Remove as much of the supernatant as possible. If desired, wash the vesicle pellet in the appropriate buffer (media, 1x PBS, etc) as needed for downstream application.

Vesicle+Particle Isolation

6 Resuspend final vesicle pellet in buffer. Store at -20 or -80 C.

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