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Extracellular vesicle isolation from bacterial cultures

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Steven Biller[1]

[1]Wellesley College

Extracellular Vesicles

Steven Biller
Wellesley College

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

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Abstract

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

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.

Equipment

Whatman Polycap TC 0.2 µm capsule filter	NAME
capsule filter	TYPE
Whatman	BRAND
6717-9502	SKU

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.