Purifying ARG

Measuring the concentration of purified ARG proteins

Estimated bench time: 60 min
Estimated total time: 13 hours

MATERIALS

- 50 mL Conical tubes
- Pipettes + tips
- Table centrifuge
- Eppendorf table centrifuge
- 2 mL Eppendorf tubes
- 1.5 mL Eppendorf tubes
- 21.5 Gauge needle
- Nanodrop
- Large cultures with ARG proteins
- PBS
- Benzoase
- BugBuster
- Ice bucket

SETUP & PROTOCOL

1. Add, of each large culture, 9 mL and 39 mL to the weighted 50 mL conical tubes.
2. Spin the cultures down at 350 * g for 4 hours at 25 °C in a table centrifuge.
3. Collect the pellets on ice and discard the supernatant. If the ARG variants produce a buoyant band of cells, the middle layer between the buoyant cells and precipitated cells needs to be removed and discarded. Weigh the tubes and calculate the weight of the pellets.
4. Resuspend the pellets in 10 mL BugBuster per 1L large culture.
5. Incubate the lysate for 1 hour at 4 °C while stirring.
6. Add 10 µL benzoase per 1L large culture.
7. Incubate for 10 minutes at 25 °C.
8. Transfer the lysate to 2 mL tubes and centrifuge them for 2 hours at 400 * g at 8 °C in the Eppendorf table centrifuge.
9. Remove the subnatant with a 21.5 gauge needle or pipette the supernatant to a 2mL Eppendorf tube.
10. Add PBS to the gas vesicles in a threefold volume excess.
11. Repeat the centrifugation, removal of subnatant and dilution with PBS two times (steps 8, 9 and 10). After this, you only have the purified gas vesicles.
12. Measure the concentrations of the ARG proteins on Nanodrop and store them in the fridge.