Mar 28, 2024

Conditioned Media Concentration with Amicon Ultra Centrifugal Filters

DOI
dx.doi.org/10.17504/protocols.io.e6nvw1px7lmk/v1

Joanna Bons[1], J P Rose[1], M A Watson[1], B Schilling[1]

[1]Buck Institute for Research on Aging

M A Watson
Buck Institute

DOI: https://dx.doi.org/10.17504/protocols.io.e6nvw1px7lmk/v1

Protocol Citation: Joanna Bons, J P Rose, M A Watson, B Schilling 2024. Conditioned Media Concentration with Amicon Ultra Centrifugal Filters. **protocols.io https://dx.doi.org/10.17504/protocols.io.e6nvw1px7lmk/v1**

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

Created: March 25, 2024

Last Modified: March 28, 2024

Protocol Integer ID: 97338

Keywords: Conditioned Media, Centrifugal Filters, Proteomics, Secretome, SASP, media concentration with amicon ultra centrifugal filter, conditioned media concentration, amicon ultra centrifugal filter, conditioned media, centrifugal filter, using centrifugal filter, concentration, cultured cell, downstream processing, complete media, serum

Abstract

Conditioned media has to be concentrated for downstream processing. To do this, complete media was removed the day before the study endpoint from cultured cells/tissue, and were washed with serum-free media. Cells/tissue were then incubated with serum-free media for 24 hours. After 24 hours, the conditioned media was collected from cultured cells/tissue and concentrated using centrifugal filters.

Materials

- Amicon Ultra-0.5 Centrifugal Filters, 3-kDa MWCO (Millipore Sigma)
- Centrifuge

Troubleshooting

1 Insert the Amicon Ultra 0.5 mL Centrifugal Filter into one of the provided microcentrifuge tubes.

2 Add up to 0.5 mL of conditioned media to the filter device and cap it.

3 Place the capped filter device into the centrifuge rotor and align the cap strap toward the center of the rotor; counterbalance with a similar device.

4 Spin the device at 12,000 × *g* for approximately 10 minutes.

5 Repeat steps 2 to 4 until the entire volume of conditioned media is concentrated down to ~50 µL.

6 Remove the assembled device from the centrifuge and separate the filter device from the microcentrifuge tube.

7 To recover the concentrated solute, place the Amicon Ultra filter device upside down in a clean microcentrifuge tube. Place in the centrifuge and align the open cap towards the center of the rotor; counterbalance with a similar device.

8 Spin for 2 minutes at 1,000 × *g* to transfer the concentrated sample from the device to the tube.

9 Continue with downstream processing or store at -80°C.