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

Conditioned Media Concentration with Amicon Ultra Centrifugal Filters

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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

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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.