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Extracellular Vesicle Isolation

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We use this protocol and it's working

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Abstract

These steps are used to isolate extracellular vesicles from primary neuronal cell cultures. It uses a combination of centrifugation and a ExoEasy Kit from Qiagen for isolation.

Extracellular Vesicle Isolation

1 Do all steps in the hood and be sterile

2 Grow Neurons and ME49 B7 parasites separately

3 Change Neuron media every 3 days and save at -80°C

4 At Day 9 of the neurons purify the parasites and infect the neurons with an MOI of 0.5

5 After infection wait 3 hours and then change half of the neuronal media

6 Continue change and saving the neuron media until the neurons die

7 Once all the supernatant is collected and filtered centrifuge at 500 x g for 15 minutes at 4°C

8 Take out the supernatant and ultracentrifuge it at 15,000 x g for 20 minutes at 4°C

9 Use the exoEasy Maxi Kit (Qiagen 76064)

10 Add 1 volume buffer XBP to 1 volume of sample and mix well by gently inverting the tube 5 time

11 Let the mixture warm up to room temperature

12 Add the sample/XBP mix onto the exoEasy spin column and centrifuge at 500 x g for 1 min at room temperature

13 Discard the flow-through and place the column back into the same collection tube

14 Add 10 mL buffer XWP and centrifuge at 4,000 rpm for 5 min at room temperature to remove residual buffer from the column

15 Discard the flow-through together with the collection tube

16 Transfer the spin column to a fresh collection tube

17 Add 400 µl of Buffer XE to the membrane and incubate for 1 min

18 Centrifuge at 500 x g for 5 min at room temperature to collect the eluate

19 Re-apply the eluate to the exoEasy spin column membrane and incubate for 1 min

20 Centrifuge at 4,000 rpm for 5 min at room temperature to collect the eluate and transfer to an appropriate tube (not supplied)

21 Aliquot out the EVs and store at -80°C

22 Run NTA on the EVs the same day before freezing EVs