Title	**Exosomes Extraction**		
Protocol Number	PARA20001	Written by	Duha A.
Adapted from	Adapted from MO Lab & CFP Lab	Version	1.0
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Before starting:
- Pre warm RPMI media

Materials:
50mL falcon tubes (x13)
Eppendorf tube
0.45 nm filter
0.2 nm filter
50mL syringe
T-175 Flasks / T-75 Flasks

Reagents:
80mL RPMI phenol red/FBS free media
160 mL PBS
2 mL HEPES

Material & reagents:
Phenol-free RPMI media
Ultra-centrifuge tubes (14mm)
50 mL conical tubes
SDM media

Protocol:

Day 1
1. Prepare a 50mL parasite culture (2.5mL parasite in 50mL SDM)

Day 3 or 4

2. Depending on the speed at which the parasite grow, after 3 or 4 days, start 800mL parasite cultures by either:
    a. Adding 200 mL SDM + 12.5 mL parasite (total of 4 T-175 flasks)
    b. **OR**
    c. Adding 400 mL SDM + 25 mL parasite (total of 2 T-175 flasks)
    d. Check the flasks daily, and shake them gently to resuspend the parasite & aid their growth

Note: T-75 flasks also work fine

Day 10 or 11 (exosome extraction)

3. Distribute the content of the culture media into 50mL conical tubes (x8)
4. Centrifuge the parasites at 2000 RPM for 10 mints.
5. Remove the media
6. Repeat the above steps until all the parasites have been collected

7. Wash the parasite pallets twice with PBS (centrifuge at 2000 RPM for 10 mints each time)
8. Collect the parasite into a total of 6 conical flasks
9. Add 13mL of prewarmed phenol-free RPMI media to each conical
10. Incubate the conical at 37°C with agitation at 40 RPM for 4 hours
11. Centrifuge the tubes at 3000 RPM for 10 mints and collect the RPMI media into new, clean tubes.
12. Filter the RPMI media twice, first using a 30 or 50 mL syringe & 45nm filter, followed by a 20/22nm filter.
13. You can store the supernatant in 4°C until the next day or continue

To continue

14. Transfer the filtered RPMI media into ultracentrifuge tubes (6 tubes in total).
15. Fill the tubes until the top (to prevent them from collapsing during centrifugation). 13 mL is a good volume.
16. Weigh the ultracentrifuge tubes + the casing using a balance. Pairs should have **exactly identical** weights (1 and 4, 2 and 5, 3 and 6), while all tubes should have roughly the same volume.
17. Spin the tubes at 100,000 xg at 4C for 1 hr.
18. After the centrifugation cycle, carefully remove most of the RPMI media, leaving behind 200-300uL. Sometime, if the concentration is high enough, you can visually see a yellowish pellet of exosomes at the bottom of the tube.
19. Collect the 300uL from all tubes into a single ultra-centrifuge tube and fill it with exosome buffer. Prepare a balance tube as directed in step 16.
20. Instead of 6, this centrifugation cycle will be for two tubes only. Spin the tubes at 100,000 xg at 4C for 1 hr.
21. Repeat step 18.
22. Pipette up and down to collect all the exosomes at the bottom of the tube.
23. Aliquot 50uL for MicroBCA, 20uL for TEM, and 20uL for NTA.
24. Store the remaining 200 uL in one eppendorf.
25. Store the samples at -80C