Title	LM-1 Infection & Stimulation with Leishmania & exosomes		
Protocol Number	CELL20001	Written by	Duha A.
Adapted from	CFP Lab & MO Lab	Version	1.2
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Protocol:

1. $5x10^5$ cells are seeded into each of the 6 well plate (with 3mL media in each well)
2. Cells are incubated overnight (16-18 hrs.)

Next day:

3. Count the parasite, remove SDM media (through centrifugation) and adjust the parasite count to a total $2x10^7$ WT L. infantum per mL (making a ratio of 20:1) in completed DMEM media.
4. Add 1mL of parasite containing DMEM to each well.
5. Add 10µg/mL of exosomes to each well
6. Incubated the cells for 6 hrs.
7. Collect the supernatant (1 mL) in eppendorfs AND wash the cells with PBS (3x)

With the supernatants:

8. Spin the eppendorfs at 3000 RPM for 5 mints to spin down the parasite.
9. Carefully collect 200uL of the supernatant (preferably from the top) and aliquot it in the Eve Technologies eppendorfs.
10. Store the supernatants at -80°C until they are sent to Eve technologies.

With the washed cells:

11. Remove PBS from the wells.
12. In the fume hood, add 1mL of TRIzol to each well. Incubate for 5 mints.
13. Collects the content of each well into an Eppendorf
14. Store the lysed cells at -80°C or continue with RNA extraction

Note: 100ng/mL LPS is used as a positive control
Note: All TRIzol manipulations are done in the fume hood.