# STANDARD OPERATION PROCEDURE (SOP)

| #1 | GENERAL INFORMATION |
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| Procedure Title | Determination of the lipid content of extracellular vesicles |
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| Creation Date | 10/02/2023 |

| #2 | PROCEDURE DESCRIPTION |
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| The lipid assay allows you to determine the lipid content of your EV sample. | |
| Duration | approximately 90-120 min (depends on the number of samples) |

| #3 | HAZARD SUMMARY |
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| Use 96% sulphuric acid in a fume hood with an acid-resistant pipette. Particular care should be taken to place acid-contaminated materials (e.g. pipette tips, paper towels) in hazardous waste containers. Use chloroform in a fume hood. Remember to use protective gloves and lab coats. | |

| #4 | STORAGE REQUIREMENTS |
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| The DOPC standard, phospho-vanillin reagent, should be stored at 4 °C. Sulphuric acid can be stored at room temperature. | |

| #5 | STEP-BY-STEP OPERATING PROCEDURE |
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1) Preparation of a lipid standard:
   a) Dissolve 1 mg DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) in 1 mL chloroform in a 2 mL volume test tube.
   b) Evaporate the chloroform at 60°C in a thermo-block under a fume hood.
   c) Add 1 mL PBS to the dried DOPC cake. 10-20 min incubation at RT may help to re-suspend all the DOPC from the surface.
   d) Vortex the tube intensively for 2-5 min at maximum speed.
   e) Sonicate with 35 kHz at 45°C for 10-20 min.
   f) Vortex the tube intensively for 2-5 min at maximum speed.
   g) The obtained liposome standard is stable at 4°C for several months.
2) Preparation of phospho-vanillin reagent:

a) Dissolve 50 mg vanillin in 50 mL of 17% phosphoric acid.
b) The dissolved phospho-vanillin reagent is stable at 4°C in dark for a couple of months.

3) Determination of lipid content of EVs:*

a) Prepare serial dilution (40 µL volume) in your EV buffer from DOPC standard (0, 0.25, 0.5, 1, 2, 4, 8, 16 µg DOPC in a test tube).
b) Add 200 µL of 96% sulfuric acid to 40 µL of DOPC liposome standards or to 40 µL EVs.
c) Briefly vortex the samples.
d) Incubate open test tubes at 90°C in a fume hood for 20 min.
e) Cool down the tubes to room temperature by placing them for at least 5 min at 4°C.
f) Briefly vortex the samples.
g) Add 120 µL of phospho-vanillin reagent to each tube.
h) Briefly vortex the tubes.
i) Transfer 280 µL of each sample to a 96 well plate.
j) Incubate 1h at 37°C allowing colour reaction to be developed.
k) Determine absorbance at 540 nm with a plate reader.
l) Calculate the lipid content of the EV samples using the absorbance at 540 nm and the equation of the standard curve.

| #6 | NOTES |
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*For determination of lipid content of EVs use test tube that previously tested. The plastic composition and possible coating or wall residue of the test tube was found to be critical for the success of the assay. We found that some test tubes, may contain surface coats that can possibly interact with components of the assay and may cause artefacts.