Lipid assay protocol for EVs

Preparation of a lipid standard:

As a lipid standard, 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) liposomes is used in 1mg/mL concentration. DOPC is purchased form Sigma-Aldrich (P6354) in lyophilised form (https://www.sigmaaldrich.com/catalog/product/sigma/p6354?lang=en&region=HU).

1. Dissolve 1 mg DOPC in 1 mL chloroform in a 2 ml volume test tube.
2. Evaporate the chloroform at 60°C in a thermo-block under a fume hood.
3. 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
4. Vortex the tube intensively for 2-5 min at maximum speed
5. Sonicated with 35 kHz (Emmi 20, EMAG) at 45°C for 10-20 min
6. Vortex the tube intensively for 2-5 min at maximum speed
7. The obtained liposome standard is stable at 4°C for several months

Preparation of phospho-vanillin reagent:

1. Dissolve 50 mg vanillin (Sigma W310727) in 50mL of 17% phosphoric acid (Sigma 79617)
2. The dissolved phospho-vanillin reagent is stable at 4°C in dark for a couple of months

Determination of lipid content of EVs

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.

1. Prepare serial dilution (40 µL volume) in your EV buffer from DOPC standard (0-0.25-0.5-1-2-4-8-16 ug DOPC in a test tube
2. Add 200 µL of 96% sulfuric acid to 40µl of liposome standards or to 40µl EVs
3. Briefly vortex the samples
4. Incubate open test tubes at 90°C in a fume hood for 20 min
5. Cool down the tubes to room temperature by placing them for at least 5 min at 4°C
6. Briefly vortex the samples
7. Add 120 µL of phospho-vanillin reagent each tube
8. Briefly vortex the tubes
9. Transfer 280µl of each sample to a 96 well plate (VWR 732-2721)
10. Incubate 1h at 37°C allowing colour reaction to be developed
11. Determine absorbance at 540nm with a plate reader
12. Calculate the lipid content of the EV samples using the absorbance at 540 nm and the equation of the standard curve

More details: JEV 2019, VOL. 8, 1565263 https://doi.org/10.1080/20013078.2019.1565263

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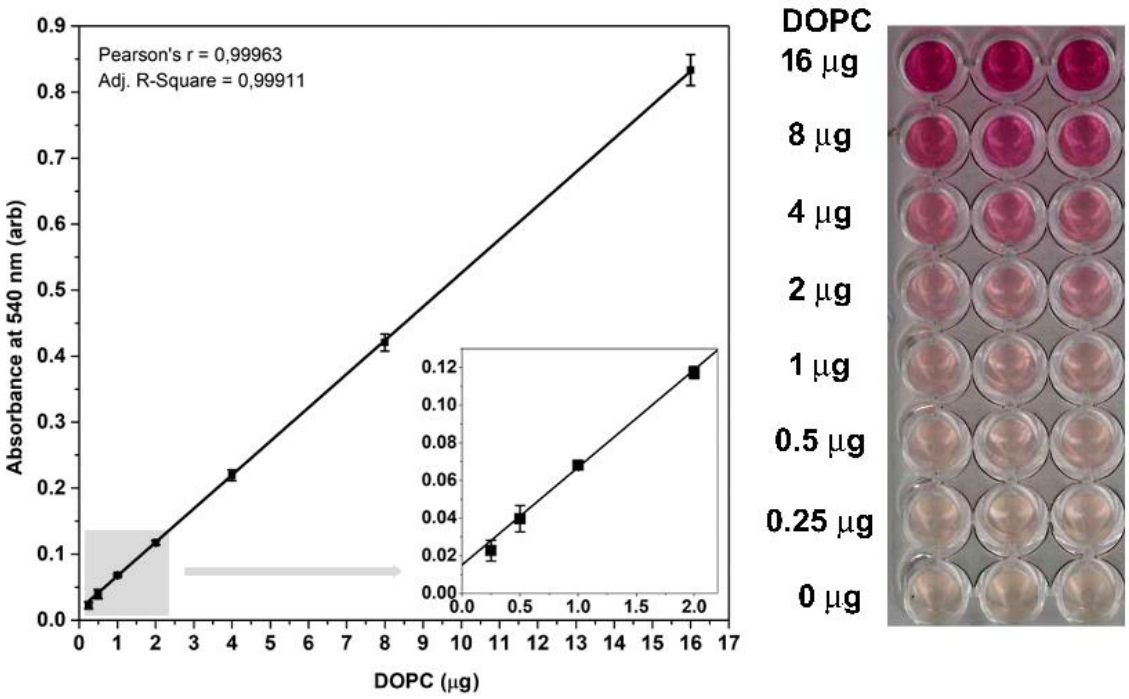
Standard curve of lipid assay

Typical standard curve with three replicates of the optimised sulfo-phospho-vanillin lipid assay using DOPC liposome standard and optimised vanillin concentration. arb: arbitrary units, error bars refer to SD.

Insert graph in the left panel highlights the standard curves between 0 and 2 µg DOPC. The right panel shows the colorimetric reaction in a 96 well plate.



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