STANDARD OPERATION PROCEDURE (SOP)

#1	GENERAL INFORMATION
Procedure Title	Mixed size EV isolation from conditioned media
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#2	PROCEDURE DESCRIPTION
This procedure is optimal if you would like to isolate medium size EVs and small size EVs together. It is a combinaton technique of differential centrifugation and size exclusion chromatography, which allows you to have less protein contamination.	
Duration	approximately 3-4 hours

#3	HAZARD SUMMARY
The qEV column contains < 0.1% sodium azide, which is harmful if swallowed or in contact with skin. The hazardousness of the samples can be judged by the user. Remember to use protective gloves and lab coats.	

#4	STORAGE REQUIREMENTS
Storage temperatures are indicated in the next chapter.	

#5	STEP-BY-STEP OPERATING PROCEDURE
1) Collect the conditioned media in a centrifuge tube. *	
2) Centrifuge: 300 g, 10 min, 20 °C.	
3) Discard the pellet, filter the supernatant with 5 µm pore size filter.*	
4) Centrifuge the filtered solution: 2000 g, 30 min, 4 °C.	
5) Discard the pellet, centrifuge the supernatant: 20.000 g, 40 min, 4 °C.	
6) Resuspend the pellet in 500 µL 0,2 µm filtered PBS.	
7) Purify further the EVs with size exclusion chromatography (qEV original-70). Follow the manufacturer's instructions. After the void volume (3 mL), collect 5 fractions of 500 µL. ***	

8) Check the EV content of the fractions by the intrinsic fluorescence of the EVs (e.g. EVs of HEK293T-palmGFP origin) or by AnnexinV labelling.
9) Pool the fractions with the highest EV content.
10) The EV sample is ready for further use. ****

#6	NOTES

*For reproducibility, it can be important to use the same tubes and the same centrifuges every time.

** Check your cell count and cell viability with a triple blue stain using a haemocytometer. The proportion of early and late apoptotic cells can be determined by AnnexinV and TO-PRO-3 staining with a flow cytometer.

*** For each EV sample, I recommend a preliminary experiment to check the EV and protein content (micro BCA) of 14 500 uL fractions after the void volume.

**** If you don't use the EVs within a day, snap freeze the EVs in liquid nitrogen and then place them at -80°C.