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Detection and Sorting of Extracellular Vesicles and Viruses using nanoFACS

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Protocol Information

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Summary: Recent advances in high resolution flow cytometry (HRFC), which show improvements in both light scatter and fluorescence sensitivity have resulted in the development of techniques that better isolate, stain and analyze single EVs(Boing et al., 2014; Groot Kormelink et al., 2016; Morales-Kastresana, Musich, Welsh, Telford, Demberg, Wood, Bigos, Ross, Kachynski, Dean, Feton, et al., 2019; Morales-Kastresana et al., 2017; Stoner et al., 2016; van der Vlist, Nolte-'t Hoen, Stoorvogel, Arkesteijn, & Wauben, 2012). Below, we describe protocols to fluorescently label EVs using CFDA-SE (hereinafter called CFSE), as well as antibodies targeted at specific EV surface proteins. We also provide guidelines for residual dye and antibody removal, appropriate data acquisition by HRFC and EV counting by HRFC. Figure 1summarizes these protocols.The EVs used in this protocol are derived from the DC2.4 cell line, and bone marrow derived dendritic cells (BMDCs). The DC2.4 cell line are immature dendritic cells (DCs) with very low expression of typical DC markers on their surface (unpublished observation and(Hargadon, Forrest, & Reddy, 2012)) and that release a morphologically homogeneous population of EVs (~130 nm in diameter). DC2.4 EVs will be used to demonstrate a CFSE staining in Basic Protocol 1, as well as being used as a negative control for antibody-based staining methods (Basic Protocol 2). Bone marrow dendritic cell (BMDC)-derived EVs are more heterogeneous in diameter (100-200 nm) and composition(Morales-Kastresana, Musich, Welsh, Telford, Demberg, Wood, Bigos, Ross, Kachynski, Dean, Felton, et al., 2019), and express DC markers such as MHC-II. BMDC EVs will be used to demonstrate antigen-specific staining with fluorochrome-conjugated antibodies in Basic Protocol 2. DC2.4 and BMDC-derived EVs were isolated by serial

ultracentrifugation, with concentration and diameter distribution characterized by NTA, as described before(Morales-Kastresana, Musich, Welsh, Telford, Demberg, Wood, Bigos, Ross, Kachynski, Dean, Feton, et al., 2019; Morales-Kastresana et al., 2017).

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