Nov 01, 2023

Quantitative Flow Cytometry (qFCM) protocols for end-to-end optimization of cross-platform extracellular vesicle studies

Cell Reports Methods

DOI
https://dx.doi.org/10.17504/protocols.io.36wgqjb5ovk5/v1

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Translational Nanobiolo...

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DOI: https://dx.doi.org/10.17504/protocols.io.36wgqjb5ovk5/v1

External link: https://doi.org/10.1016/j.crmeth.2023.100664

Collection Citation: Sean M Cook, Vera A Tang, Joanne Lannigan, Jennifer C. Jones, Joshua A Welsh 2023. Quantitative Flow Cytometry (qFCM) protocols for end-to-end optimization of cross-platform extracellular vesicle studies. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqjb5ovk5/v1

Manuscript citation:
Cook S, Tang VA, Lannigan J, Jones JC, Welsh JA. Quantitative flow cytometry enables end-to-end optimization of cross-platform extracellular vesicle studies. Cell Rep Methods. 2023 Dec 18;3(12):100664. doi: 10.1016/j.crmeth.2023.100664.

Protocol status: Working
We use this collection and it's working

Created: January 30, 2023

Last Modified: November 01, 2023

Collection Integer ID: 76090

Keywords: quantitative flow cytometry, extracellular vesicle, common extracellular particle, fcm fluorescence, cytometry, automated small particle optimization, light scatter detector settings for ep analysis, fcm optimization of fluorophore panel selection, frameworks such as miflowcyt, small particle optimization, fluorophore panel selection, fcm for ep analysis

Funders Acknowledgements:
NIH
Grant ID: ZIA BC011502
NIH
Grant ID: ZIA BC011503
NIH
Grant ID: 4UH3TR002881-03

Disclaimer

This protocol summarizes key steps for a specific type of method, which is one of a collection of methods and assays used for EV analysis in the NCI Translational Nanobiology Section at the time of submission of this protocol. Appropriate use of this protocol requires careful, cohesive integration with other methods for EV production, isolation, and characterization.

Abstract

Flow cytometry (FCM) is a common extracellular particles (EPs), including viruses and extracellular vesicles (EVs), characterization method. Frameworks such as MIFlowCyt-EV exist to provide reporting guidelines for metadata, controls, and
data reporting. However, tools to optimize FCM for EP analysis in a systematic and quantitative way are lacking. Here, we demonstrate a cohesive set of methods and software tools that optimize FCM settings and facilitate cross-platform comparisons for EP studies. We introduce an automated small particle optimization (SPOT) pipeline to optimize FCM fluorescence and light scatter detector settings for EP analysis and leverage quantitative FCM (qFCM) as a tool to further enable FCM optimization of fluorophore panel selection, laser power, pulse statistics, and window extensions. Finally, we demonstrate the value of qFCM to facilitate standardized cross-platform comparisons, irrespective of instrument configuration, settings, and sensitivity in a cross-platform standardization study utilizing a commercially available EV reference material.

Disclaimer

This protocol summarizes key steps for a specific type of method, which is one of a collection of methods and assays used for EV analysis in the NCI Translational Nanobiology Section at the time of submission of this protocol. Appropriate use of this protocol requires careful, cohesive integration with other methods for EV production, isolation, and characterization.

Files

SEARCH

Protocol

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Resource 1: Scatter Detector Setting Incrementation for FCMPASS

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Resource 2: Fluorescence Detector Setting Incrementation for FCMPASS

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Resource 6: rEV Fluorescent Detector Setting Incrementation

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