



NOV 02, 2023

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

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>

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

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

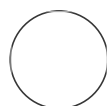
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Translational Nanobiology Section



Jennifer Jones

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.

Created: Jan 30, 2023

Last Modified: Nov 02, 2023

COLLECTION integer ID:
76090

Funders

Acknowledgement:

NIH

Grant ID: ZIA BC011502

NIH

Grant ID: ZIA BC011503

NIH

Grant ID: 4UH3TR002881-03

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.

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FILES

Protocol



NAME

✓ Resource 1: Scatter Detector Setting Incrementation for
FCMPASS

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✓ Resource 2: Fluorescence Detector Setting Incrementation for
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Resource 3: SSC Collection Optics and Calibration

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Resource 4: rEV Serial Dilution

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Resource 5: rEV Scatter Detector Setting Incrementation

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Resource 6: rEV Fluorescent Detector Setting Incrementation

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Resource 7: rEV immunophenotyping

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