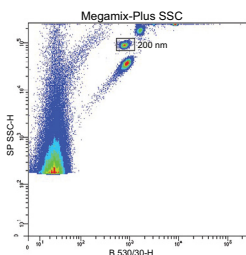


Flow cytometric analysis of extracellular vesicles

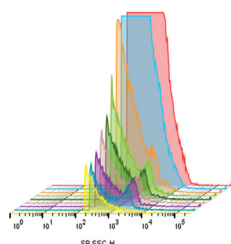
with BD FACSymphony™ A1 Cell Analyzer,
BD FACSDiva™ Software, FlowJo™ Software
and Rosetta Calibration Plugin



Fine tune your small particle detector (SPD)

STEP 1: Prepare your BD FACSymphony™ A1 Cell Analyzer using standard small particle beads

- Prepare SPD bead mixture
- Align SPD using Picomotor Application (if needed)
- Adjust SP SSC voltage to place bead peaks on target



Ensure reproducible low background noise

STEP 2: Perform start-up procedure to reduce background noise

- Run BD® Detergent Solution Concentrate
- Run sample diluent and check background noise

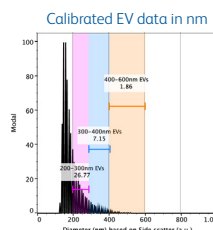
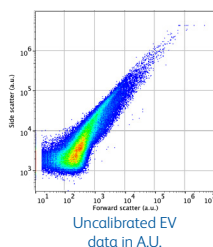
3.1 Buffer only*	3.5 Single-stained controls*
3.2 Buffer with reagents*3.6	Procedural controls*
3.3 Unstained controls*	3.7 Serial dilution*
3.4 Isotype controls	3.8 Detergent-treated EV samples

Welsh JA, et al. *J of Extracell Vesicles*. 2020;9(1):1713526.

Acquire your EV samples and explore the power of “OR” thresholding

STEP 3: Apply proper controls, calibration and thresholding strategy

- Use “OR” thresholding for SP SSC and relevant fluorescent parameters for maximum information
- Consider running serial dilution series to check for absence of swarming
- Use *MiFlowCyt-EV* framework for publication to guide you on assay controls and SP SSC and fluorescence calibration



Measure Rosetta Calibration beads and your EV samples

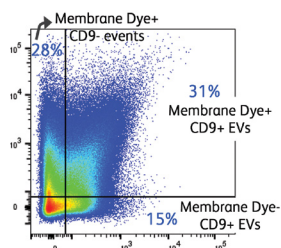
STEP 4: Optional for EV size calibration

- Run Rosetta Calibration beads and your EV samples at the same scatter detector voltage and gain
- Configure the threshold and voltage of the scatter channel so that you detect the smallest beads in the mixture while retaining maximum dynamic range



Flow cytometric analysis of extracellular vesicles

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Analyze your EV data

STEP 5: Take advantage of the analysis power of BD FACSDiva™ Software, FlowJo™ Software and the Rosetta Calibration Plugin

- Output files from recommended calibration software compatible with BD FACSDiva™ and FlowJo™ Software for further analysis
- Use the Rosetta Calibration Plugin to derive calibrated size measurements of your EV samples



Still need help?

- Follow troubleshooting guidelines in the BD FACSymphony™ A1 Cell Analyzer User's Guide
- Contact your BD support team for assistance

To learn more about the BD FACSymphony™ A1 Cell Analyzer or the BD® Small Particle Detector, contact your BD sales representative.

Get started with the Rosetta Calibration Plugin today!

Learn more about our free plugins at <https://www.flowjo.com/learn/flowjo-university>

Download Rosetta Calibration Plugin at: <https://www.flowjo.com/exchange/#!/plugin/profile?id=61>

Rosetta Calibration webinar: <https://flowjo.wistia.com/medias/92eyr9axmf>

More at: <https://www.exometry.com/products/rosettacalibration>

Plugins are free with a FlowJo™ Software license

*User should follow routine maintenance process and run CS&T before SPD procedure
Class 1 Laser Product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

BD Life Sciences, San Jose, CA 95131, USA

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