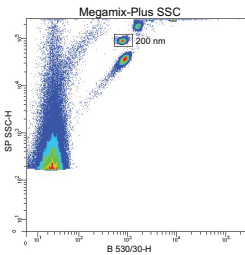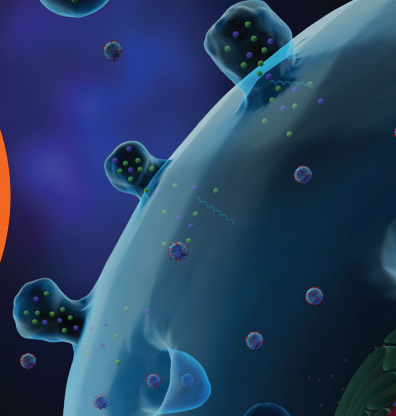# Analyze extracellular vesicles (EVs) on the BD FACSymphony™ A1 Cell Analyzer
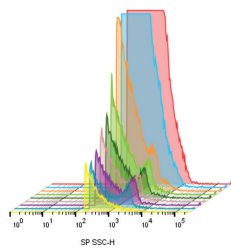
Standardized workflow for consistent results

Fine tune your small particle detector (SPD)

STEP 1: Prepare your instrument 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. Journal of Extracellular 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 *MiFlowCyt-EV* framework for publication to guide you on assay controls and SP SSC and fluorescence calibration
- Use "OR" thresholding for SP SSC and relevant fluorescent parameters for maximum information
- Consider running serial dilution series to check for absence of swarming
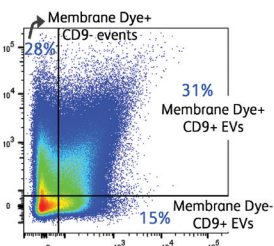
Analyze your EV data

STEP 4: Take advantage of the analysis power of BD FACSDiva™ and FlowJo™ Software

- Output files from recommended calibration software compatible with BD FACSDiva™ and FlowJo™ Software for further analysis

Still need help?

- Follow troubleshooting guidelines in the 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.

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

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BD Life Sciences, San Jose, CA 95131, USA

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