The SOP For Antibody-Labeled Exosome Detection by the Exoplorer Nano-flow Cytometer

Content

1. Use and Cleaning of SEC Column

1.1 Equilibration

1.1.1 Take out the required SEC column, fix it vertically, and secure the waste collection tube beneath the column.

1.1.2 Carefully remove the bottom cap of SEC column, then gently remove the top cap.

1.1.3 Allow the storage buffer to start running through the column under gravity.

1.1.4 Flush the column with 3 column volumes of DI water (1 column volume: 3 mL).

1.1.5 Equilibrate the column with 5 column volumes of pre-equilibrated PBS buffer at room temperature.

1.2 Sample Collection

1.2.1 Load the prepared sample (150 μL) onto the loading frit and immediately start collecting the buffer volume. The column will stop flowing when all of the sample has entered the loading frit. Label this collection tube as F1.

1.2.2 Add 170 μL of PBS buffer to the column, continue collecting the buffer volume, and label this tube as F2. Continue the elution process by repeating this step for subsequent fractions.

1.2.3 It is recommended to collect fractions F6-F9, as these are expected to contain the target sample.

1.3 Regeneration

1.3.1 Clean the column with 5 column volumes of PBS buffer.

1.3.2 Rinse the column with 2 column volumes of 0.5 M NaOH.

1.3.3 Flush the column with 5 column volumes of DI water to remove residual NaOH.

1.3.4 Flush the column with 2 column volumes of 20% ethanol.

1.3.5 Finally, add 20% ethanol above the loading frit, tightly seal the top and bottom caps, and store at room temperature.

2. Flow Cytometry Antibody Labeling of Exosomes

2.1 Sample Pretreatment (Optional)

2.1.1 Centrifuge samples at 1,500 g for 10 min to remove cell debris.

2.1.2 Re-centrifuge the supernatant at 10,000 g for 10 min and collect the supernatant.

2.1.3 Purify the sample.

2.1.4 Filter the sample using a 0.22 μm filter.

2.2 Sample Dilution

2.2.1 Dilute sample with PBS buffer to a concentration of approximately 5×10^8 particles/mL.

2.3 Sample Labeling

2.3.1 Take 150 μL diluted sample and stain it with antibodies, mixing thoroughly.

Recommended antibody dosage (Optional)

Optional Conjugated dyes	Antibody amount (μg)
PE	0.04-0.2
PE/Cyanine7	0.04-0.2
FITC	0.04-0.4

2.3.2 Incubate at 37℃ in the dark for 30 min.

2.4 Removal of Free Antibodies

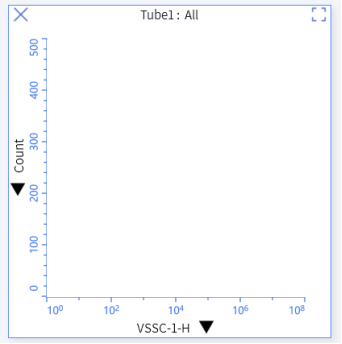
2.4.1 Refer to the sample collection steps described in Section 1.2.

3. Sample Data Acquisition, Analysis, and Result Export

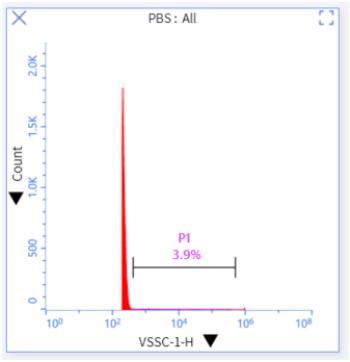
3.1 Sample Data Acquisition and Analysis（Take the PE-conjugated antibody as example）

3.1.1 Click **Experiment Management** > **New Experiment**.

3.1.2 Click **Add A Tube** in the tube panel to create a tube, and create a Histogram Plot in the Plot and analysis panel.



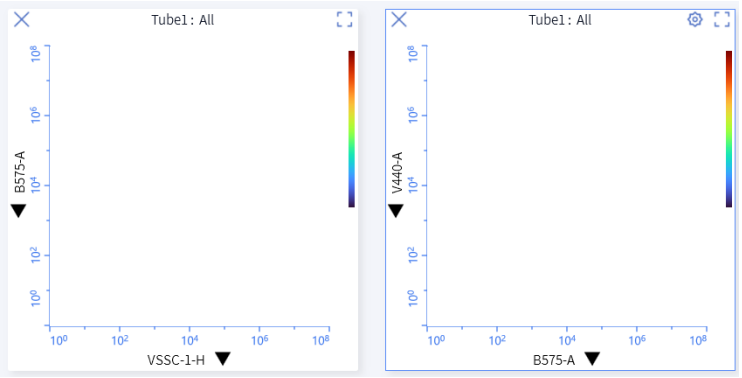
3.1.3 Buffer Control Group: Place the sample buffer (e.g., PBS buffer) into the sample rack and click the **Start** button. Set the P1 gate in the acquired histogram to exclude the instrument's background noise. Calculate the product of the EPS and the percentage of events within the P1 gate (EPS × P1 percentage). If this value is below 50, it indicates that the buffer background meets the requirements and can be used for subsequent experiments.



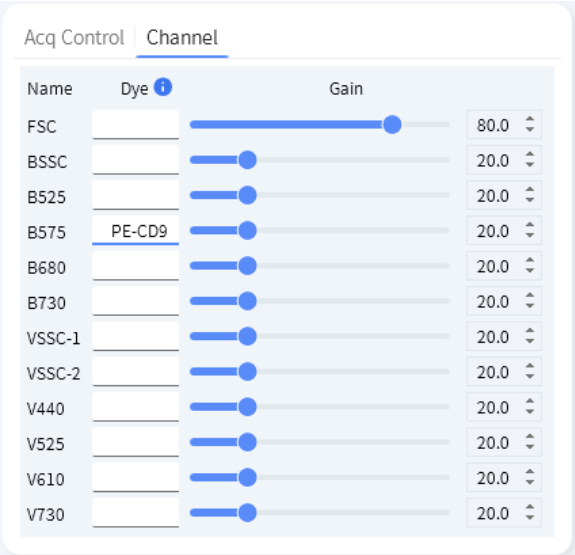
3.1.4 Click **Add A Tube** button in the tube panel to create a new tube, and create a new Pseudocolor Plot in the Plot and analysis panel.

Optional Pseudocolor Plot 1: X-axis: VSSC-1-H, Y-axis: B575-A.

Optional Pseudocolor Plot 2: X-axis: B575-A, Y-axis: V440-A (or another channel that is not B575-A).

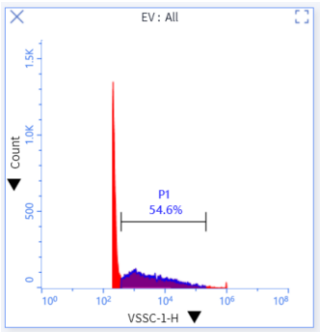


3.1.5 Click **Channel** to adjust the gain of each photodetector in every channel, and enter antibodies or dyes used for each channel.

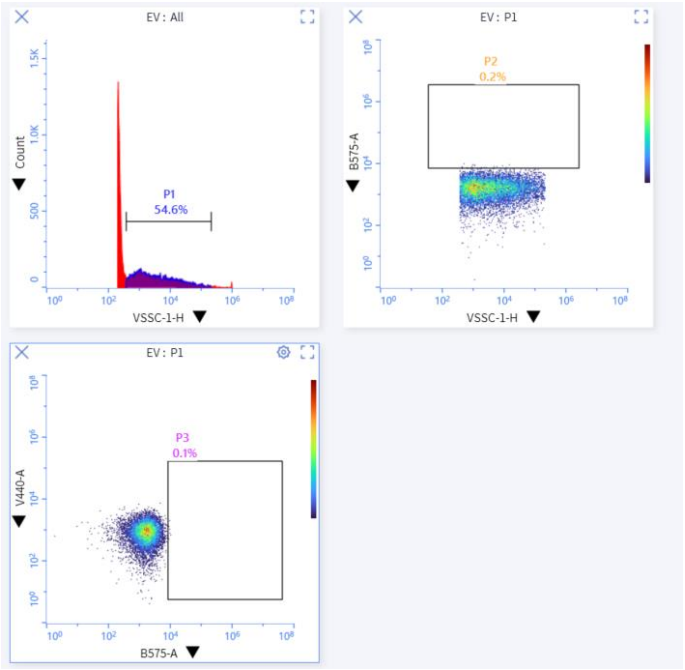


3.1.6 Negative Control Group: Place the sample (unstained) into the sample rack and click the **Start** button.

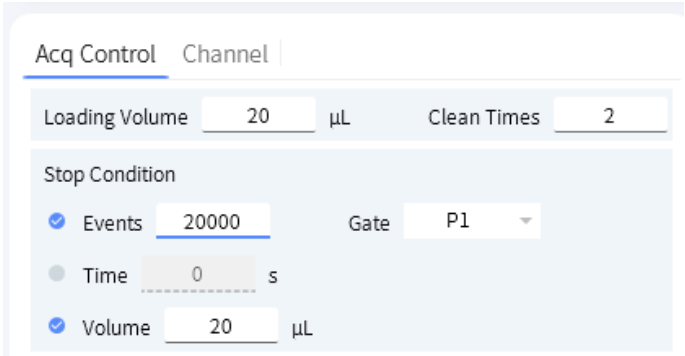
3.1.7 Adjust the P1 gate on the VSSC-1-H histogram to encompass of the target sample population.



3.1.8 Set the P2 or P3 gate: After selecting the P1 gate in the Pseudocolor Plots, set the P2 or P3 gate.

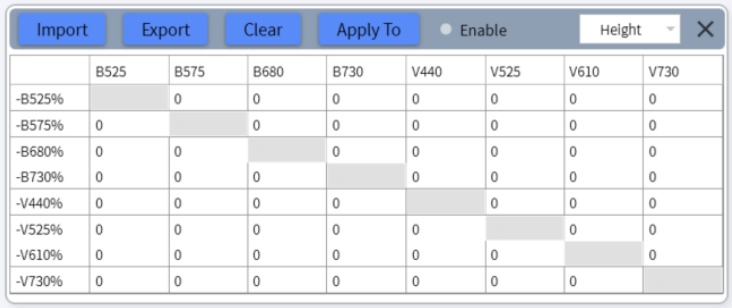


3.1.9 Disable all the stop conditions except for Events, and set P1 gate and Events to 20,000. Click **Record** button.



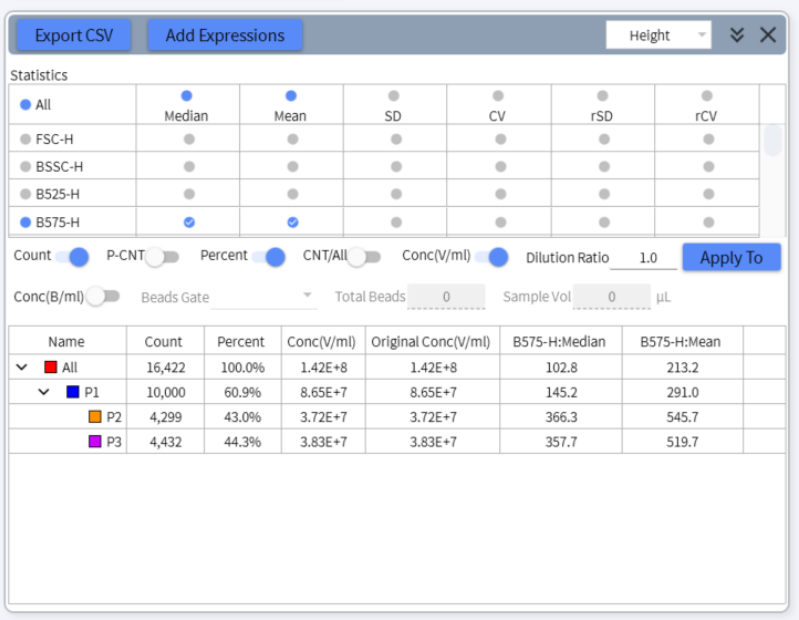
3.1.10 Isotype control or experimental group: Place the sample into the sample rack, click the **Start** button, and record the data.

3.1.11 Adjust compensation: Click **Compensation** in the tube panel to adjust channel compensation.



	B525	B575	B680	B730	V440	V525	V610	V730
-B525%		0	0	0	0	0	0	0
-B575%	0		0	0	0	0	0	0
-B680%	0	0		0	0	0	0	0
-B730%	0	0	0		0	0	0	0
-V440%	0	0	0	0		0	0	0
-V525%	0	0	0	0	0		0	0
-V610%	0	0	0	0	0	0		0
-V730%	0	0	0	0	0	0	0	

3.1.12 After acquisition stops, click **Statistics** button to open the Statistics window. Click **Median** or **Mean** for B575-H. The lower part of the window displays the median/mean fluorescence intensity for the P1/P2/P3 gates, which can be used for statistical analysis.

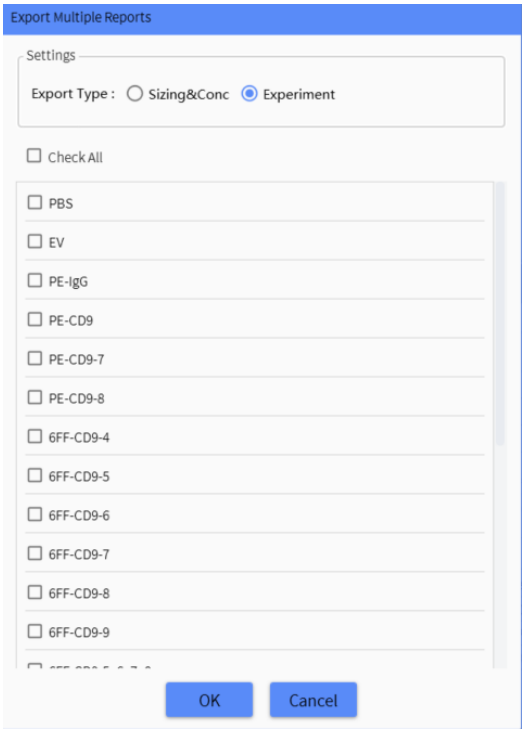


Name	Count	Percent	Conc(V/ml)	Original Conc(V/ml)	B575-H:Median	B575-H:Mean
All	16,422	100.0%	1.42E+8	1.42E+8	102.8	213.2
P1	10,000	60.9%	8.65E+7	8.65E+7	145.2	291.0
P2	4,299	43.0%	3.72E+7	3.72E+7	366.3	545.7
P3	4,432	44.3%	3.83E+7	3.83E+7	357.7	519.7

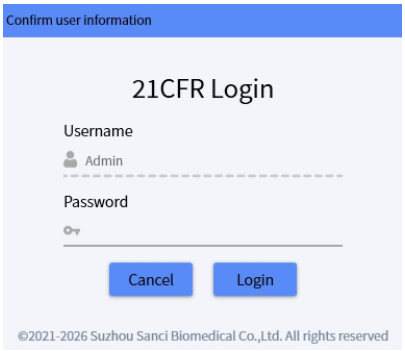
3.2 Export Experiment Report

3.2.1 Click **Multi Export** > **Export report** in the tube panel.

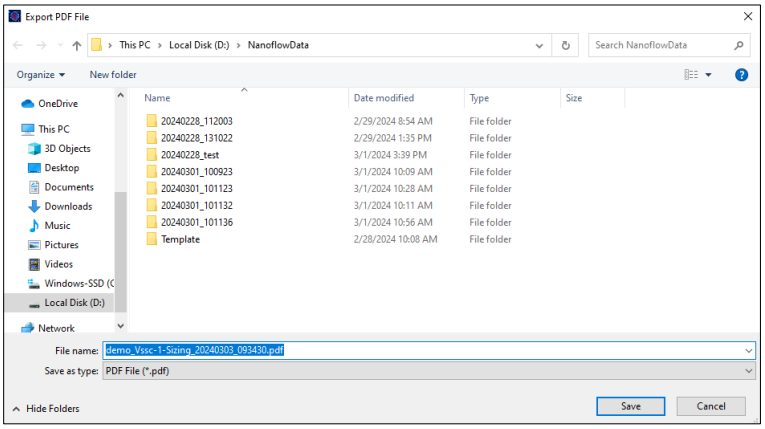
3.2.2 On popup **Export Multiple Reports** page, set Export Tyes as **Experiments**, select the tubes that need to been exported, then click **OK** button.



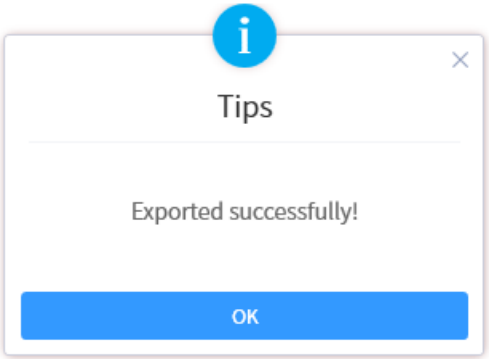
3.2.3 In the Confirm user information window, enter the password and click **Login**.



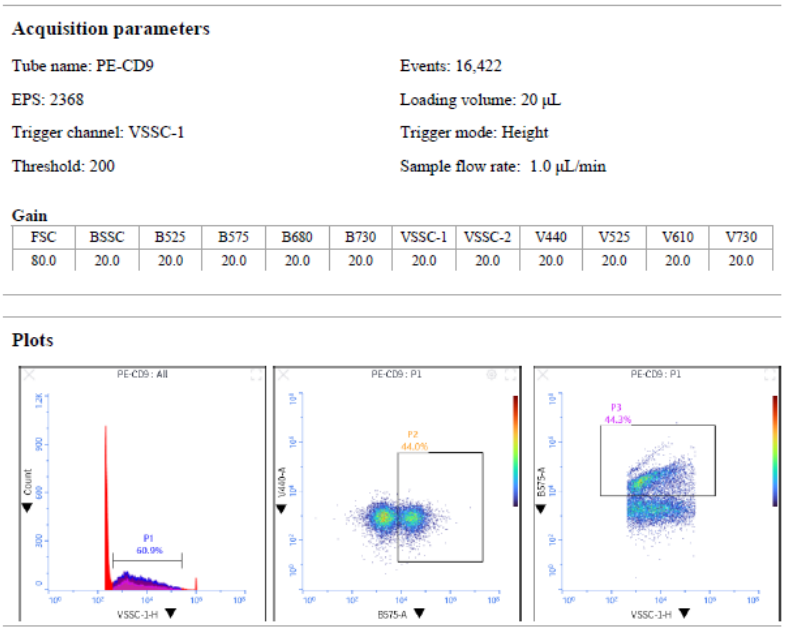
3.2.4 In the opened window, you can change the file name, specify save path and then click **Save** to save it.



3.2.5 When the Tips window (Exported successfully) appears, it means the experiments' report has been exported to the specified file folder successfully.



3.2.6 The experimental report is in PDF format and contains the necessary collected data.



Acquisition parameters

Tube name: PE-CD9 Events: 16,422

EPS: 2368 Loading volume: 20 µL

Trigger channel: VSSC-1 Trigger mode: Height

Threshold: 200 Sample flow rate: 1.0 µL/min

Gain

FSC	BSSC	B525	B575	B680	B730	VSSC-1	VSSC-2	V440	V525	V610	V730
80.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0

Plots

3.3 Export FCS file

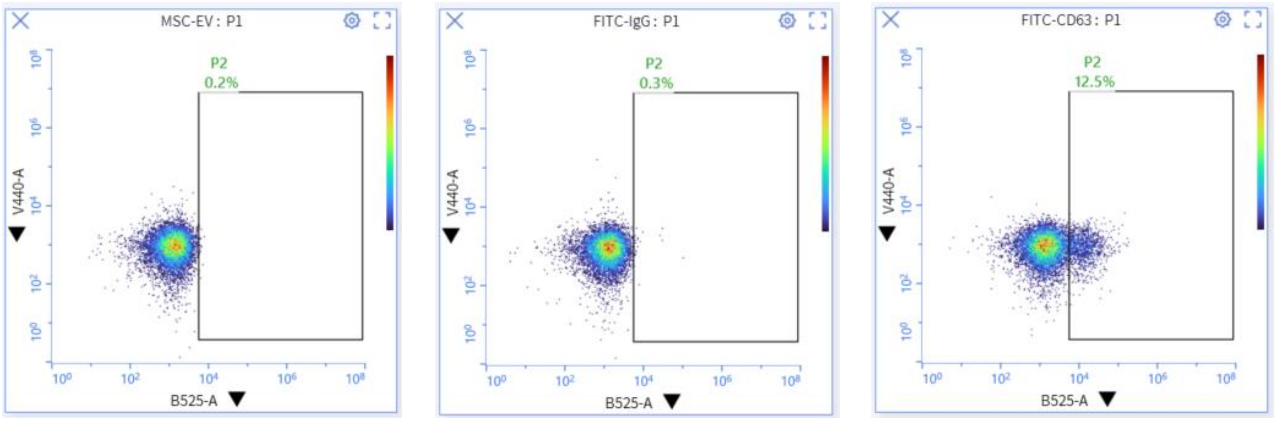
3.3.1 Click **Multi Export** button and select **Export FCS File**.

3.3.2 Select the tubes and the file save path in the opened prompt. You can analyze data independently in flow cytometry software such as FlowJo.

4. Examples of Exosome Antibody Labeling

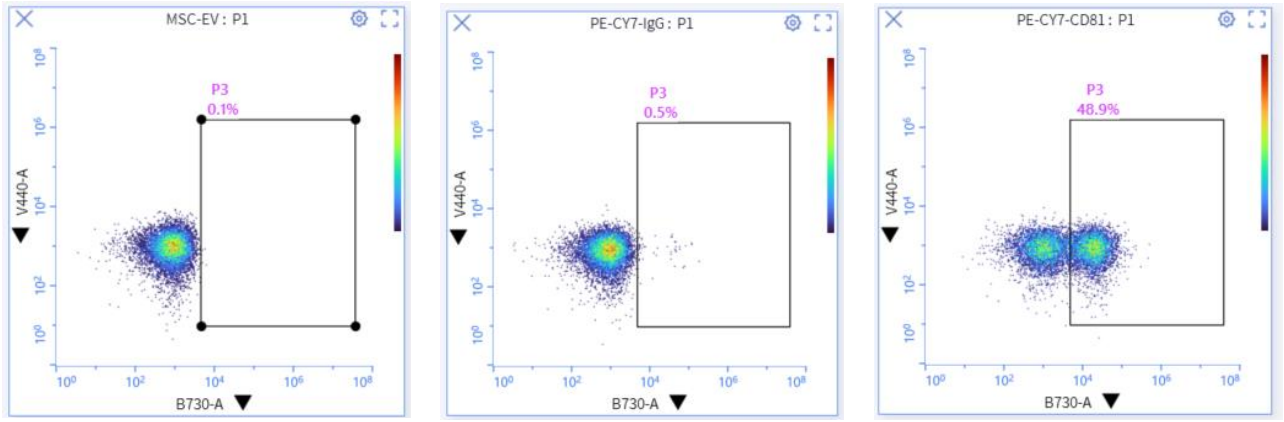
4.1 Labeling of MSC-Derived Exosomes with FITC-Conjugated Anti-CD63 Antibody

Negative Control | Isotype Control | Experimental Group



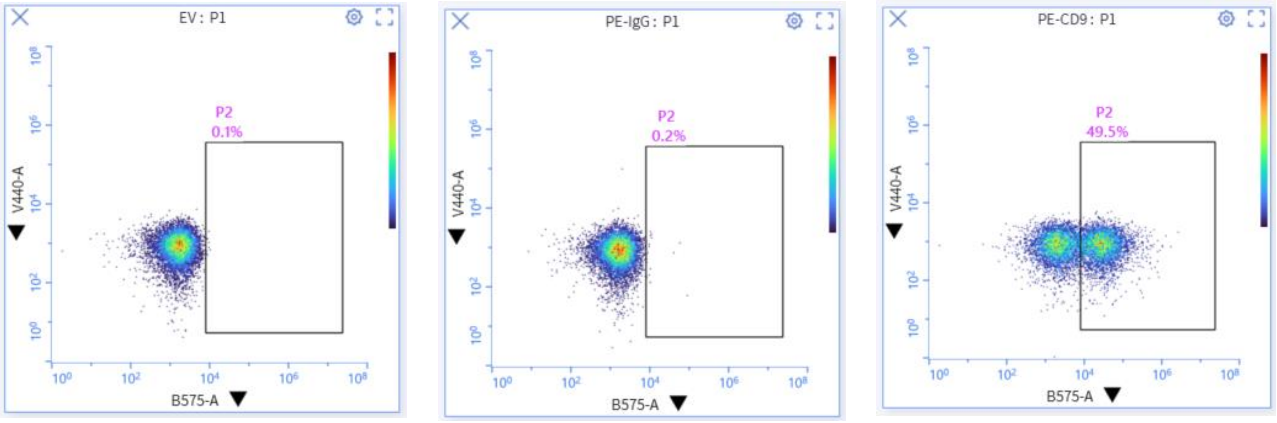
4.2 Labeling of Stem Cell-Derived Exosomes with PE/Cy7-Conjugated Anti-CD81 Antibody

Negative Control | Isotype Control | Experimental Group



4.3 Labeling of MSC-Derived Exosomes with PE-Conjugated Anti-CD9 Antibody

Negative Control | Isotype Control | Experimental Group



4.4 Multiplex Antibody Characterization of MSC-Derived Exosomes