

## Overview

This guide provides general recommendations for common sample types to ensure optimal performance. Additional optimization might be needed depending on the input materials and the target nanoparticles.

## Pre-processing of Input Materials

Sample	Recommendation
Culture medium with up to 10% serum	<ul style="list-style-type: none"> <li>Centrifuge at 500xg for 10min to remove cells and debris.</li> <li>Filter through 0.22µm PES filter (for nanoparticles &lt;200nm) or appropriate filters for the target nanoparticles.</li> </ul>
Plasma/Serum	<ul style="list-style-type: none"> <li>Centrifuge at 10,000xg for 10min at 4°C and transfer supernatant to a new tube.</li> <li>Dilute 20 times with sterile 1XPBS (without Ca<sup>2+</sup> or Mg<sup>2+</sup>)</li> <li>Filter through 0.22µm PES filter (for nanoparticles &lt;200nm) or appropriate filters for the target nanoparticles.</li> </ul>
Other bodily fluids with no more than 2mg/ml total protein concentration	<ul style="list-style-type: none"> <li>Filter through 0.22µm PES filter (for nanoparticles &lt;200nm) or appropriate filters for the target nanoparticles.</li> </ul>
Other bodily fluids with higher than 2mg/ml protein concentration	<ul style="list-style-type: none"> <li>Dilute with sterile 1XPBS (without Ca<sup>2+</sup> or Mg<sup>2+</sup>) to lower the protein concentration to 2mg/ml or below.</li> <li>Filter through 0.22µm PES filter (for nanoparticles &lt;200nm) or appropriate filters for the target nanoparticles.</li> </ul>

## Wash Buffer Preparation

The table below provides recommendations to wash buffer volumes for different cassettes. If higher purity is desired, more wash buffer can be used, though processing time will increase accordingly.

Cassette	Standard Protocol	For Higher Purity
VanoFlow™-EXO-01	35ml	50ml
VanoFlow™-EXO-02	45ml	60ml
VanoFlow™-EXO-03	150ml	225ml

## Post-processing of Purified Nanoparticles

If desired, the purified nanoparticles can be further processed for various applications or purposes. The table below provides recommendations to commonly employed protocols.

Purpose	Recommendation
Sterilization	<ul style="list-style-type: none"> <li>Filter through 0.22µm or 0.45 µm PES filter.</li> <li>Note: Non-PES filter may result in higher nanoparticle loss</li> </ul>
Further particle concentration	<ul style="list-style-type: none"> <li>For 30–200nm particles, use 100kD MWCO PES concentrating column and centrifuge at 500xg until desired volume is reached.</li> <li>Note: The concentrating speed depends on the starting concentration of nanoparticles.</li> </ul>