



VanoFlow[®]-EXO-20

EV Isolation Cassette

Instruction Manual

Doc Num: IM-VF-EXO-20 Rev A



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1. Product Information

1.1 Kit Overview

Name:	VanoFlow®-EXO-20 EV Isolation Cassette
Part Number:	VF-EXO-20
Function:	To be used with NanoEX™ Bio-nanoparticle Isolation System for isolation of Extracellular Vesicles (EV)
Size Cutoff:	>30 nm
Shelf Life:	Two (2) years from the date of manufacturing
Storage:	Store at 4–30 °C in a dry environment, protected from moisture, direct sunlight, and extreme temperatures
Reusability:	Up to 3 total uses, depending on sample type, sample volume, and operating conditions
Input Volume:	100 to 600 mL
Output Volume:	~35 mL
Sterilization Method:	Ethylene Oxide (ETO)

1.2 Kit Contents

Name	Qty
VanoFlow-EXO-20 cassette	1
Tubing set bag (First Use + 2x Reuse + Cleaning)	1
Sample collection bottle (100mL) x 3, included in VanoFlow-EXO-20 tubing set bags	3
NFC tags (3x Purification + 3x Cleaning)	6
Part storage box	2

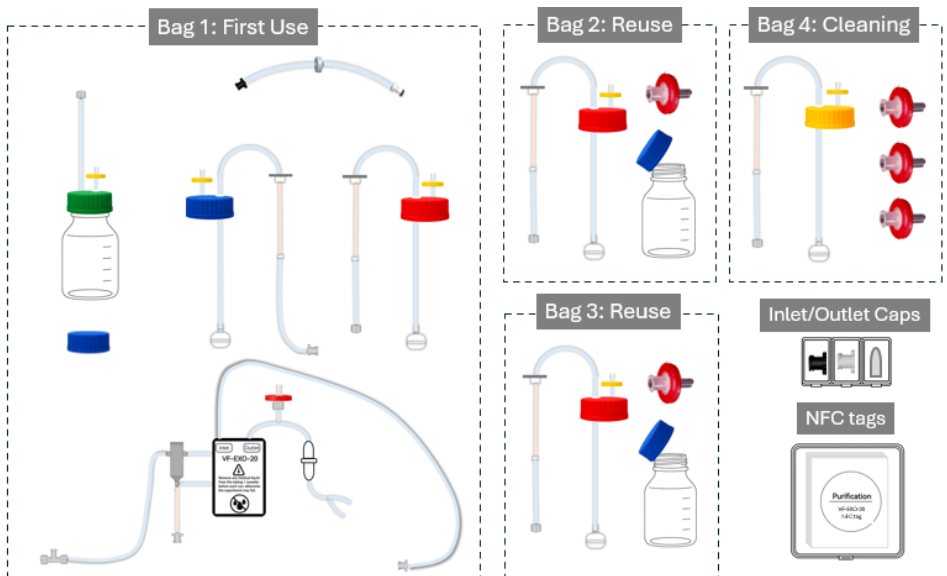
1.3 Materials Provided

- VanoFlow® Cassette



⚠ Important: The **outlet of the cassette** consists of a small, barbed fitting and may be damaged if mishandled. Handle with care.

- Tubing Set and Accessories



- Tubing Set and Accessories (Cont.)

Buffer Feed
Cap (blue)
Assembly



Sample Feed
Cap (red) Assembly
X3
(Single-Use)



Cleaning Feed
Cap (yellow)
Assembly



Collection
Cap (green)
Assembly



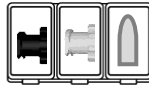
Collection Bottle
X3
(Single-Use)



Venting Valve
X5
(Single-Use)



Cap Storage Box
X1



NFC Tag Storage
Box
X1



Process Tubing
Set
X1



Cassette Waste
Tubing
X1



- About NFC Tags

A valid **Near Field Communication (NFC) Tag** is required to initiate any NanoEX™ instrument protocol. Each kit includes two types of NFC tags, which are shape-coded to prevent misuse and ensure reliable identification.

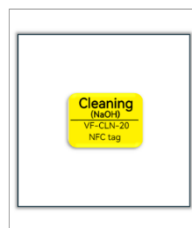
- **Purification NFC Tags (x3)**

- **Appearance:** round tag labeled with Purification *VF-EXO-20*
- **Displayed process name:** *VanoFlow-EXO-20*
- **Intended use:** Use when performing **EV purification with new cassette or reused cassette after cleaning.**



- **Cleaning NFC Tags (x3)**

- **Appearance:** rectangular tag labeled with Cleaning *VF-CLN-20*
- **Displayed process name:** *VanoFlow-CLN-20*
- **Intended use:**



1. When performing **an automated cassette cleaning** cycle on a previously used cassette.
2. (Optional) Prior to the first use, perform a cleaning cycle to minimize residual endotoxins in the cassette before EV purification.

 **Important NFC Tag Handling Information**


- **Do not remove the NFC tag at any time during processing.** Removal of the NFC tag will cause the run to pause or abort.
- **Each NFC tag is single-use only.** Once sample loading has started, the NFC tag is permanently consumed and must be discarded after the run

- NanoEX™ Accessories (Provided in the accessory kit)

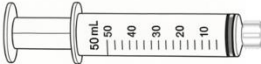


The Cleaning Accessory Set (Reusable)

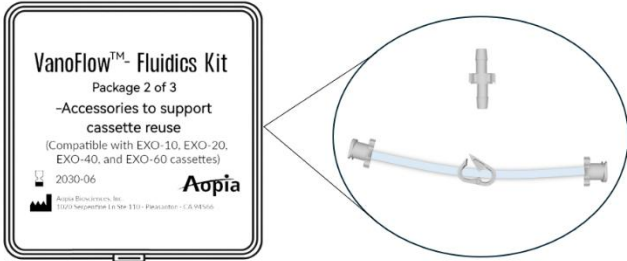
100mL NaOH Cleaning Collection Bottle



Syringe (50 mL)



VanoFlow-Fluidics Kit (Package 2 of 3)



The kit package is labeled: VanoFlow™ Fluidics Kit, Package 2 of 3, -Accessories to support cassette reuse, (Compatible with EXO-10, EXO-20, EXO-40, and EXO-60 cassettes), 2030-06, Aopia, Aopia Biosciences, Inc., 100 Neumann in the 310+ Pasadena - CA 91106.

⚠ Important: The Cleaning Accessory Set is **NOT single-use** and should NOT be discarded. It will be reused for future cleaning cycles.

1.4 Materials Required but Not Provided

Name	Qty (for each run cycle)
0.05M HCl (for storage)	40 mL
0.5M NaOH (for cleaning)	200 mL
PBS or TBS buffer	360 mL
70–75% Ethanol or Isopropyl alcohol (IPA)	200 mL
DI water or distill water	200 mL
GL-45 bottles (for Sample, Buffer, and cleaning solution)	3
Quart-size (or larger) Zip lock bag (for tubing storage)	1

1.5 Safety Information – Chemical Hazards

WARNING:

Sodium hydroxide (NaOH) and hydrochloric acid (HCl) solutions are **highly corrosive** and may cause **severe skin burns, eye damage, and respiratory irritation** if mishandled.

▪ Required Personal Protective Equipment (PPE)

When handling NaOH, HCl, or contaminated components, the user must wear:

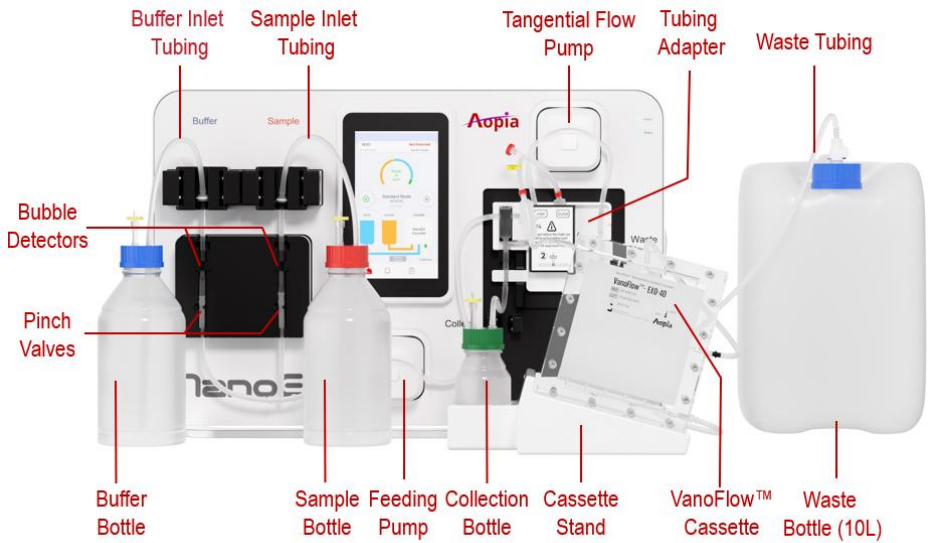
- Chemical-resistant gloves (e.g., nitrile)
- Safety goggles or face shield
- Laboratory coat or protective clothing

▪ Chemical Handling and Neutralization

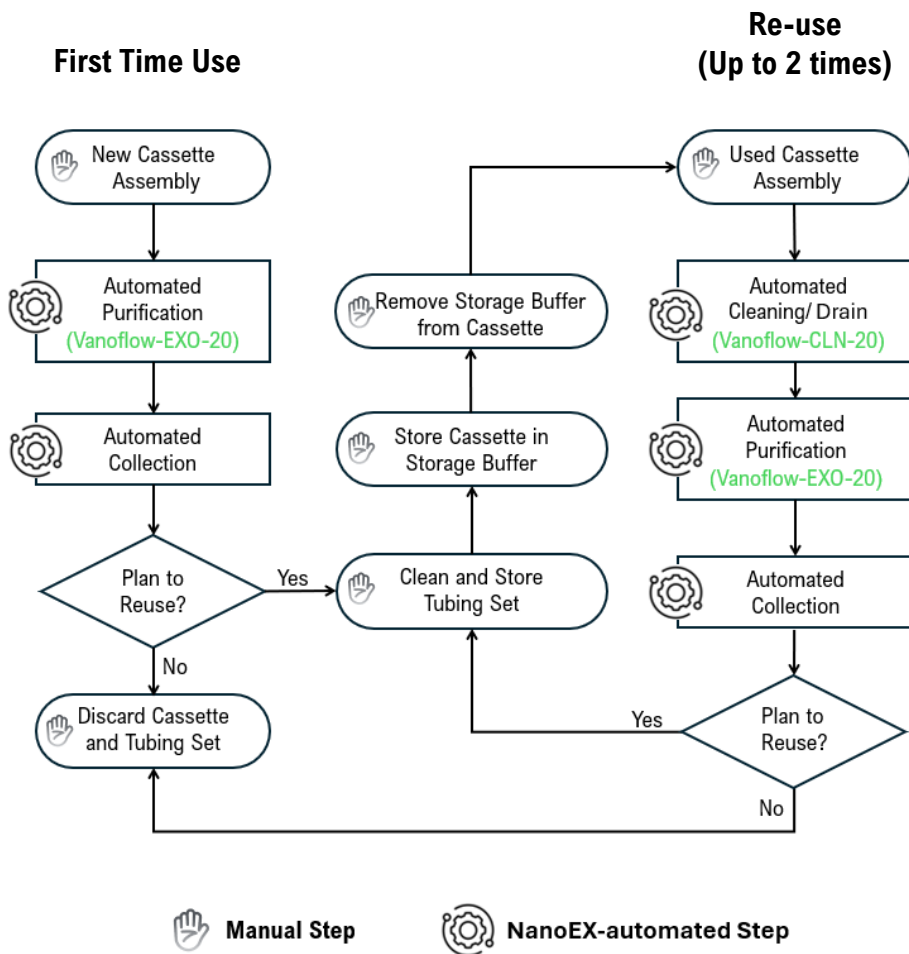
- NaOH cleaning of the cassette must always be followed by a buffered wash (e.g., PBS or TBS, ≥ 50 mM) to fully neutralize residual alkalinity.
- Do NOT use non-buffered solutions (e.g., DI water alone) for NaOH neutralization.
- Refer to manufacturer's safety data sheet (SDS) for more information.



2. System Overview



3. Workflow Overview




■ Reuse Considerations

To minimize the risk of cross-contamination, it is **recommended** that the same cassette be reused with **the same sample type or equivalent sample matrix** whenever possible.

■ Cassette Reuse Timing and Storage Requirements

- If automated cassette cleaning is performed within 48 hours after the previous use, the cassette storage buffer loading step may be skipped.

- If automated cleaning is not performed within 48 hours, the cassette must be filled with storage buffer prior to storage.
- After completion of an automated cleaning cycle, EV purification must be performed within 48 hours.
- Between uses, the cassette must remain capped at all ports to prevent contamination and drying, and must be stored at 4–8 °C.

 **Note:** Failure to follow these timing and storage requirements may compromise membrane performance and cassette reusability.

4. Before You Start

4.1 System Readiness Checklist

Before starting a run, verify that the system meets the following requirements.

- Software requirements
- Verify that the instrument is operating with:
 - **NanoEX Software Version V3.5.4 or later (earlier model) or V5.5.4 or later (newer model). See Software Compatibility below**, and
 - **Protocol Profile V2.6.1 or later.**
- Software compatibility (by last four digits of the serial number):
 - Use Software V3.x.x for serial numbers \leq 0030
 - Use Software V5.x.x for serial numbers \geq 0031.
- The Software Version is displayed on the **Home Screen**.
- The Protocol Profile version can be found under **Settings**.
- The latest NanoEX Software and Protocol Profile are available on the official Aopia website: <https://aopiabio.com/software>
- Waste Bottle
 - Verify the waste bottle is **empty** before starting a run.
 - Place the bottle at the **same height as the instrument** to maintain proper drainage.



Note: Failure to meet the system readiness requirements may affect system performance.

4.2 Sample and Wash Buffer Preparation

For detailed sample pre-treatment procedures, refer to the **Sample Preparation Guide**.

- It is recommended that samples not be frozen prior to processing, as freezing may cause EV damage and protein aggregation, which may compromise purification efficiency.
- Pre-filter samples through 0.2µm or 0.45µm depending on the desired EV sizes.

4.3 Determine the Appropriate Processing Mode

The system provides **two purification modes** and **one cleaning mode**:

- EV Purification (Process Name: **VanoFlow-EXO-20**)
 - **Standard Purification Mode** – This mode is **recommended for most sample types**. Always start with this mode when processing a new or uncharacterized sample type.
 - **Accelerated Purification Mode** – This mode is intended only for samples with very low protein content (< 0.1 mg/mL) and without complex macromolecules.
- Cassette Cleaning (Process Name: **VanoFlow-CLN-20**)
 - **Cleaning Mode** – This mode is designed **exclusively** for cleaning the VanoFlow Reusable Cassette at an elevated flow rate. This mode must not be used for purification.

The screenshot shows a user interface titled "Select Processing Mode". It contains three selectable options, each in a rounded rectangular box:

- Standard Purification Mode** (highlighted with a red border):
 - (Recommended) Optimized for high-purity processing.
 - Suitable for most samples, including plasma, serum, and conditioned medium with/without exosome-depleted FBS.
- Accelerated Purification Mode**:
 - Processes samples at a faster speed with reduced purification efficiency.
 - Suitable only for samples with minimal impurities.
- Cleaning Mode**:
 - Cleaning the Vanoflow® Reusable Cassette at a higher flow rate (not suitable for purification).
 - The Vanoflow® Reusable Cassette can be used several times if proper cleaning and storage procedures are followed.

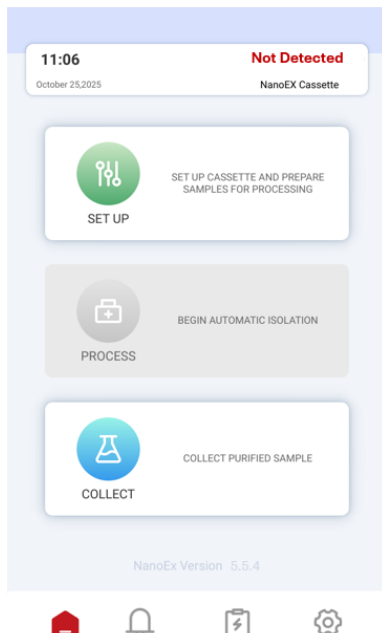
At the bottom of the interface is a blue "Select" button.

5. Processing Procedure – First Time Use

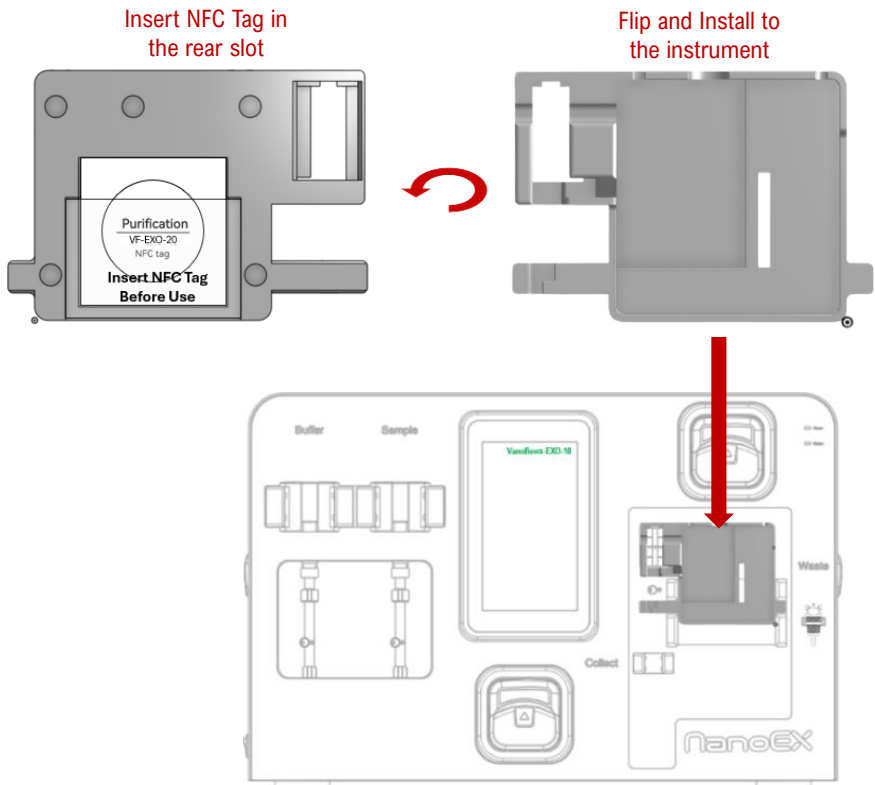
5.1 Step 1 - Cassette Assembly

5.1.1 Install NFC tag and Tubing Adaptor

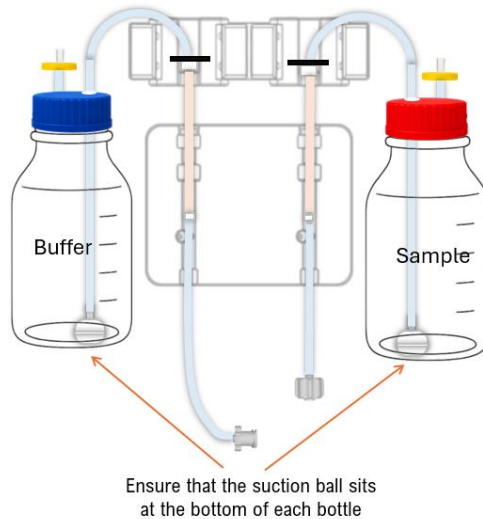
1. Power on the NanoEX system. After system initialization is complete, click **Start** Button to enter the **Home Page**.



2. Insert the **Purification NFC Tag (VF-EXO-20)** into the rear slot of the Tubing Adapter.
 - DO NOT remove the NFC tag from the liner (back paper).
3. Flip the **Tubing Adapter** to the correct orientation and install it onto the NanoEX instrument as shown in the diagram below.
4. Verify cassette detection on the touchscreen:
 - A **green cassette name** should appear in the upper-right corner of the screen, indicating successful detection.
 - If **“Not Detected”** or **“Unknown”** is displayed, refer to the **Troubleshooting** section before proceeding.



5.1.2 Install Sample and Buffer Bottles



1. Ensure **GL-45 bottles** (not provided) are used for both **sample** and **buffer**.
 - The **buffer bottle** should have a minimum volume of 360 mL.
2. Open the **Tubing Set Bag (First Use)**.
 - Install the **Red Cap** onto the empty **sample bottle**.
 - Install the **Blue Cap** onto the empty **buffer bottle**.
 - Place the **buffer bottle on the left** and the **sample bottle on the right**.
3. Adjust the **Suction Ball** position by gently pulling the tubing so that the suction ball rests at the **bottom of the bottle**, with the **Inlet Port facing downward**.

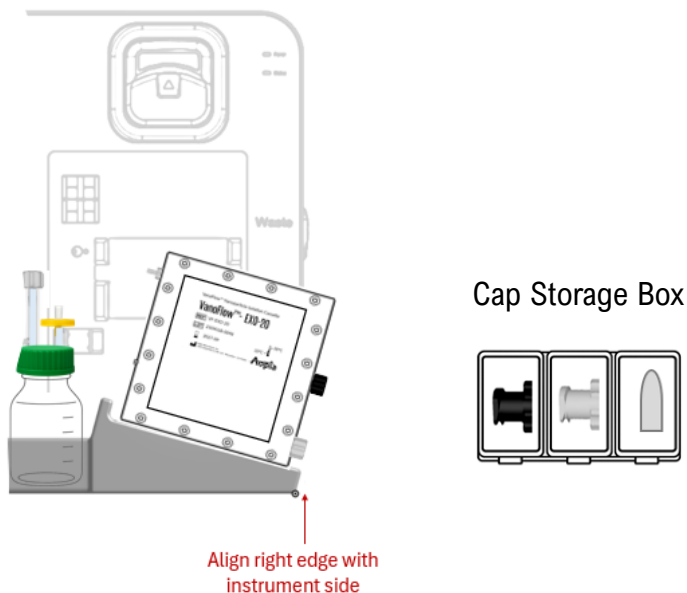
⚠ Important: Incorrect positioning of the suction ball may increase dead volume and compromise performance.

⚠ Important: Do **not** use cap tubing assembly from a different cassette model (e.g., using **EXO-40** cap tubing assembly with an **EXO-20** cassette). The cap tubing assembly must match the installed cassette; otherwise, the process will not proceed.

5.1.3 Place Cassette and Collection Bottle

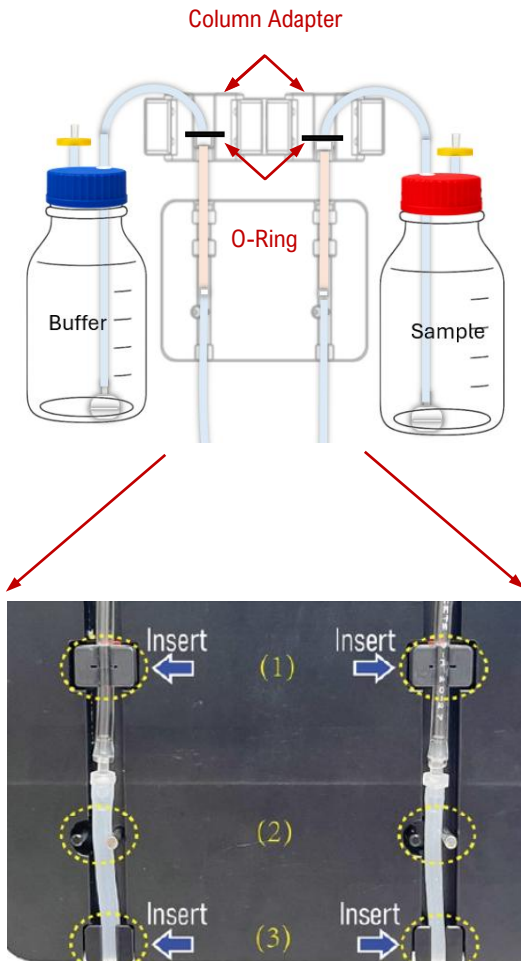
1. Place the **Cassette** onto the **Cassette Stand** with the **label facing the user**, ensuring the **black waste outlet** is positioned on the **right side**.
 - Place the cassette closer to the instrument on the cassette stand.
- ⚠ **Important:** If using a previously used cassette, **remove the storage buffer** from the cassette before proceeding.

Note: Do not spray IPA or ethanol directly onto the cassette surface, as this may damage the cassette.
2. Position the **Cassette Stand** so that **its right edge is aligned with the right side of the instrument**, and place the stand close to the instrument.
3. Remove the **Yellow Filter Caps** from the **inlet** and **waste ports** of the cassette and discard them. **Do NOT** discard any other **Caps**. Store the caps in the provided three-chamber storage box for later use.
4. Place the **collection bottle** (provided), fitted with a **Green Cap**, into the **collection bottle holder** on the Cassette Stand.



5.1.4 Install Bottle Tubing

1. Ensure that the **removable Column Adapters (2X)** are installed.
2. Load the **black rubber O-rings** into the **Column Adapters** from the **top opening**.



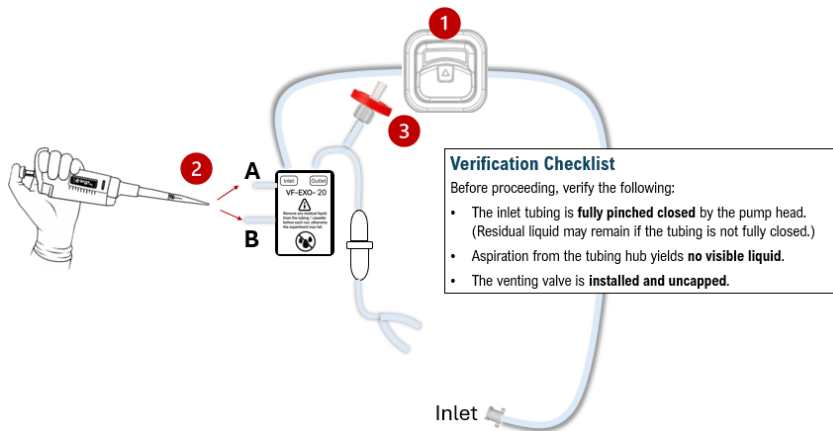
3. Insert the **clear tubing** into the **(1) Detector**
4. Insert the **translucent tubing** into **(2) the Valve** and **(3) the Fitting**

5.1.5 Install Process Tubing Set

5.1.5a Empty the Tubing Hub

Follow the procedure below when re-using a processing tubing set

- For a **previously used tubing set**, perform the following steps to **remove any residual liquid from the tubing hub** before proceeding.
- Failure to remove residual liquid **may result in improper operation of the automated process**.



Procedure

Step 1.

Use the pump head to **pinch (close) the inlet tubing**.

This step **must** be completed before proceeding.

Step 2.

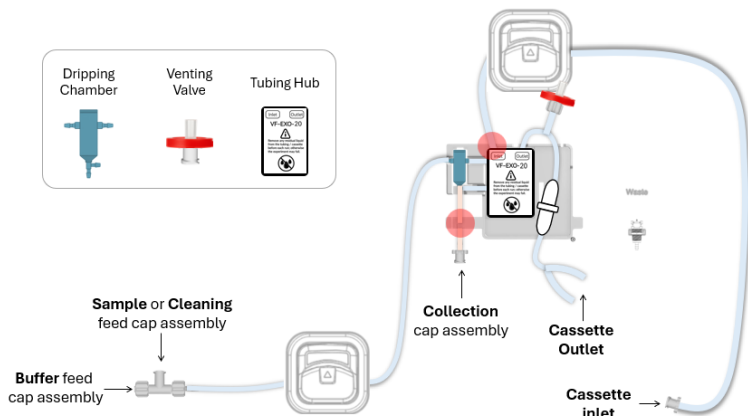
Using a pipette with a **1 mL tip**, **fully insert the tip into tubing ends A and B to form a seal**, then aspirate any remaining liquid from **tubing ends A and B** until no liquid can be withdrawn.

Step 3.

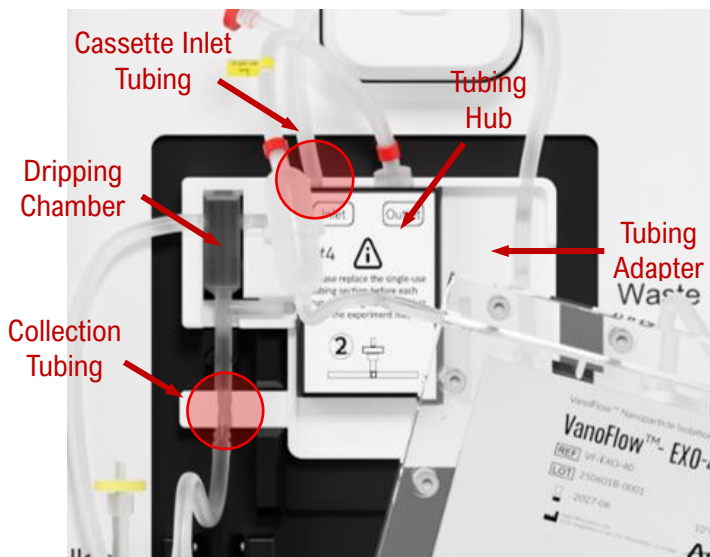
Replace the venting valve with a **new, venting valve**.

Important:

- Hold the tubing hub **vertically** during aspiration to facilitate complete liquid removal.

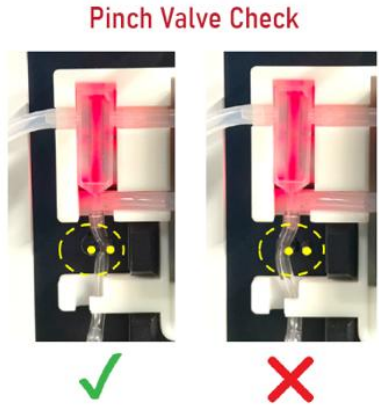


1. Attach the **Dripping Chamber** and **Tubing Hub Assembly** to the Tubing Adapter by inserting each component into its designated slot.
2. Press the **Dripping Chamber** firmly into the sensors located behind the adapter until it is fully seated.
3. Insert the **Collection Tubing** and **Cassette Inlet Tubing** into their designated fittings, as indicated by the **red circles**.



4. Verify that the **Collection Tubing** is positioned between the two pins of the **pinch valve**, as indicated by the yellow circles.

⚠ WARNING: If the tubing is not properly pinched, **the process will fail.**



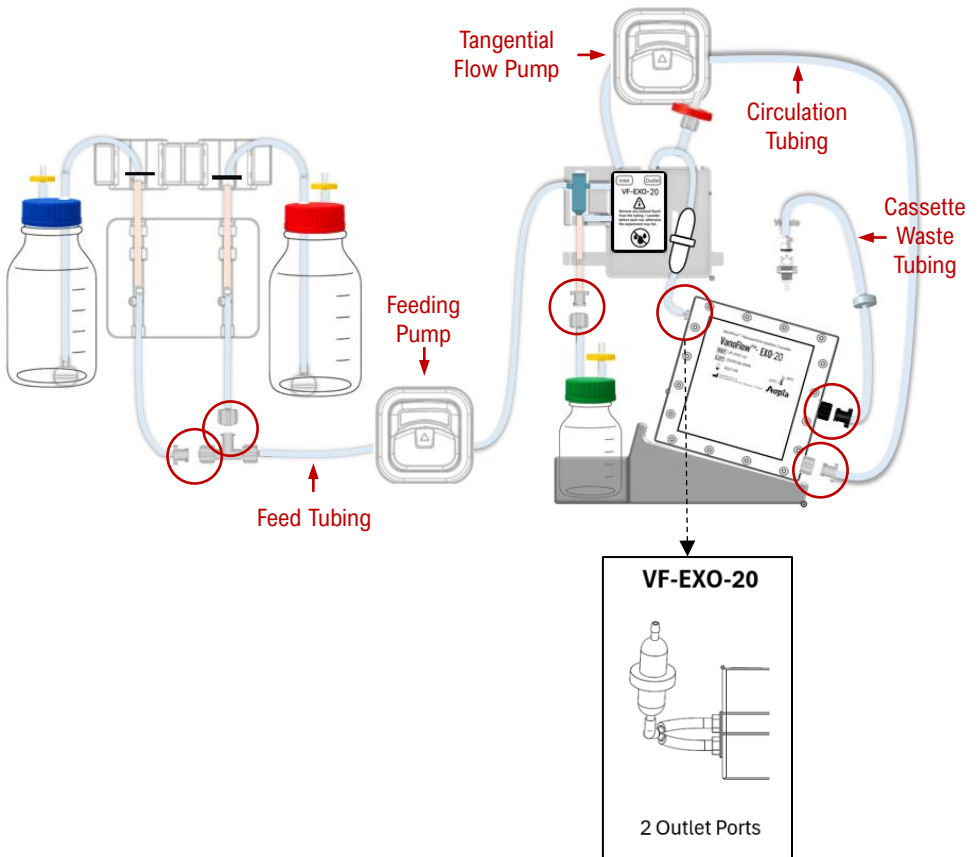
5.1.6 Install Cassette Waste Tubing

1. Press the thumb latch on the **Waste Connector** until it clicks.
2. Insert the **Cassette Waste Tubing end** into the **Waste Connector**.
3. Place the **Waste Container** at the same level as the instrument.



5.1.7 Connect Tubing

1. Connect the tubing using **Luer Lock** or **barbed fittings**, following the schematic indicated by the **red circles**.
2. Tighten all **Luer Lock connections** securely by hand.
3. Ensure that the **sample inlet tubing** is positioned vertically after connecting it to the **T-connector**.
4. Verify that all tubing paths are **free of kinks** or **sharp bends** to allow unobstructed flow.
5. The **VanoFlow-EXO-20** cassette has **two (2)** outlet tubing/outlet port connections. Ensure that **all** connections are securely connected.



5.1.8 Install Tubing to Pumps

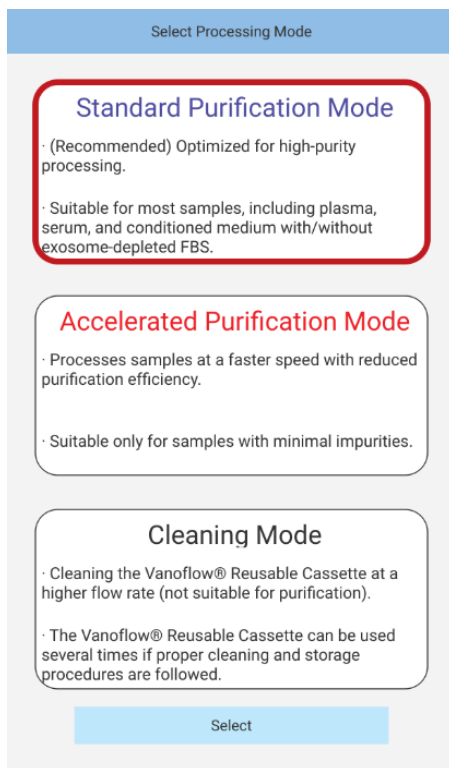
1. Install the **feed tubing** onto the **feeding pump**, and install the **cassette circulation tubing** onto the **tangential flow pump**.
2. To install the tubing, place **the tubing** into the **V-shaped inner brackets**, then push down the top covers to close the pump heads. Do not stretch the tubing during installation.



5.2 Step 2 - Automated Processing

5.2.1 Setup Check

1. Verify that the **Cassette Name** is correctly displayed in the **top-right corner of the touchscreen**, then click the **“Set Up”** to open the **Processing Mode Selection** page.
2. Select **“Standard Purification Mode”** (recommended).
3. Follow the on-screen instructions to verify each key setup step before starting the run.



5.2.2 Input Sample Volume

When prompted, enter the **sample name** and **sample volume**.

The instrument will then calculate **an estimated processing time** on the next screen.




IMPORTANT:

- The entered sample volume **must not deviate by more than $\pm 10\%$** from the actual sample volume.
- **Do not remove sample** from the bottle during processing.
- Failure to follow these instructions **may result in processing failure**.
- The sample volume input function is intended to **improve process reliability** by establishing appropriate timing references for the processing.
- This function does not **automatically adjust sample loading volume** based on the entered volume.

Please enter the sample information

Sample name
(a-z, 0-9, "_" or "-" only)

Sample volume mL
(integer only)


 **Warning:** The entered sample volume must not differ from the actual sample volume by more than **10%**; otherwise, the experiment may fail.


Please confirm the sample volume

Sample Volume : mL

Recommended Buffer Volume : mL

Estimated Processing Time :

 Experiment may fail if the entered sample volume deviates from the actual volume by 10%

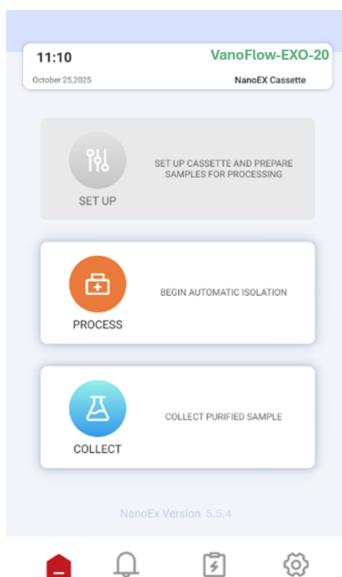
 Experiment may fail if the sample is removed during processing.

5.2.3 Add Sample and Buffer

1. When prompted by the on-screen instructions, add **the sample (up to 600 mL)** and **buffer (360 mL)** to their corresponding bottles, then tighten the caps securely.

Note:

- **Do not** add sample or buffer through the **yellow vent filter**.
 - The vent filter is intended for **gas venting only**. Wetting the filter will **significantly hinder solution loading**.
2. After the sample and buffer have been added, select “**Done**” to return to the Home page. The “**Process**” button will become active. Select “**Process**” to proceed.



⚠ IMPORTANT:

- **Do not overload the sample** - Adding a sample volume that significantly exceeds the cassette’s rated capacity may cause severe membrane fouling, which can compromise purification performance, hinder effective cleaning, and reduce the number of reuse cycles.


- **Use the recommended buffer volume** - Insufficient buffer may lead to poor purification performance.
- When samples are stored cold and processed at room temperature—**particularly culture medium samples**—it is recommended to either:
 - Place the sample and buffer bottles in an insulated container with ice packs to maintain a low temperature during the run (Refer to Appendix 1 - **Sample Cooling Guide**), or
 - Allow the sample to equilibrate **to within 5°C difference from room temperature** before starting the run.

Rapid temperature increase may lead to **gas bubble formation**, which can falsely trigger the sensor and result in **incomplete sample loading**.

Common Installation Errors and Consequences

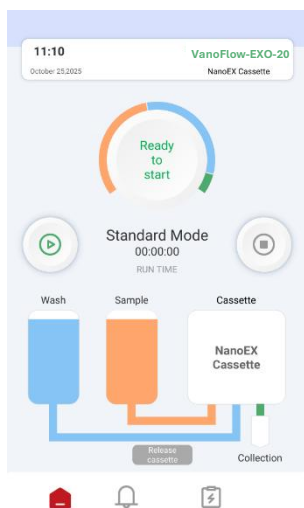
Common Installation Error	Consequence
Failure to empty the Tubing Hub before each run, as described in Section 5.1.5a, “Empty the Tubing Hub.”	The pre-test may fail, and sample loading may be incomplete or result in excessive elution volume
Failure to replace the red venting valve with a new one before each run.	
Tubing is not properly positioned between the two pins of the pinch valve, resulting in incomplete pinching.	The processing will fail
Failure to cap the venting valve during collection (Page 32).	Incomplete sample collection

5.2.4 Begin Purification




1. Click **“Process”** to enter the main processing page.
2. Click the **Start** icon () to begin automated purification.

Reminder:

- During the initial phase of the run (approximately the first **12 minutes**, prior to sample loading), the system performs **automated pre-checks** to verify correct installation and detect potential warnings or errors.
- Users are advised to **monitor the system during this initial period**. Once sample loading begins and no warnings are present; the run is designed to proceed **without further user intervention** under normal operating conditions.



Functional Icons

-  Return to the Home Page
-  Warning Messages
-  Run Logs

IMPORTANT:

- If sample or buffer needs to be added or removed, the processing must be paused first. **Removing samples during processing is not permitted and will result in process errors.**

5.2.5 Aborting a Run and Restart

In the event of an **unexpected condition** or **pre-test failure**, the run **may be aborted and restarted** following the guidance below.

▪ **Case 1: Run Aborted *Before* Sample Loading Begins**

(During buffer pre-loading and before the sample valve opens)

- The NFC tag remains marked as “**NEW**”, and **no NFC tag replacement is required**.
- **This is the preferred restart point** if installation or sensor errors occur.

Restart Procedure:

1. Abort the run.
2. **Empty the buffer bottle** (required).
3. Navigate to the **Home** page and perform a **Collection** cycle to empty the cassette.
4. Refill the buffer bottle with **fresh buffer**.
5. Restart the process using the **same cassette** and the **same NFC tag** after correcting any installation errors.

▪ **Case 2: Run Aborted *After* Sample Loading Begins**

(The sample valve has opened)

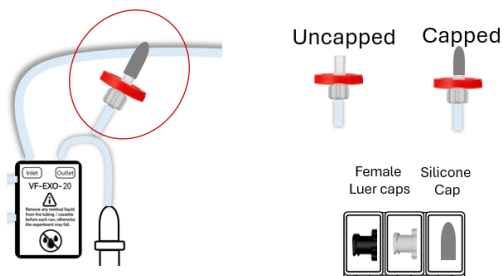
- The NFC tag will be marked as “**USED**”, and a **new NFC tag must be used**.


Restart Procedure:

1. Abort the run.
2. **Empty the buffer bottle** (required).
3. Navigate to the **Home** page and perform a **Collection** cycle to collect the sample already loaded into the cassette.
4. Combine the collected sample with the remaining sample in the **sample bottle**.
5. Refill the buffer bottle with **fresh buffer**.
6. Restart the process using the **same cassette** and a **new NFC tag** after correcting any installation errors.

5.2.6 Collection

1. After automated purification is completed, a pop-up window “**Processing Completed**” will appear and an alarm will sound. Click “**OK**” to return to the **Home** page.
2. Click “**Collect**” and **confirm** to enter the Collection screen. Follow the on-screen instructions to verify readiness before starting the collection run.



3. Before starting collection, cap the **venting valve** with the provided **silicone cap**. This step facilitates proper drainage from the dripping chamber.
4. Select the **Start** icon () to begin collection.
5. After collection is completed, a “**Collection Completed**” message will appear and an alarm will sound.



Important:

- If the collected sample volume is lower than expected, **inspect the dripping chamber for residual sample**.
- If residual sample is present, carefully disassemble the dripping chamber and tubing hub and **recover the remaining purified sample using a pipette** (refer to section 5.1.5a **Empty the Tubing Hub**) to maximize recovery.

6. Remove the collection bottle containing the purified sample from the **Green Collection Cap Assembly**. Recap it with the **Blue Cap** provided in the Tubing Set Bag.

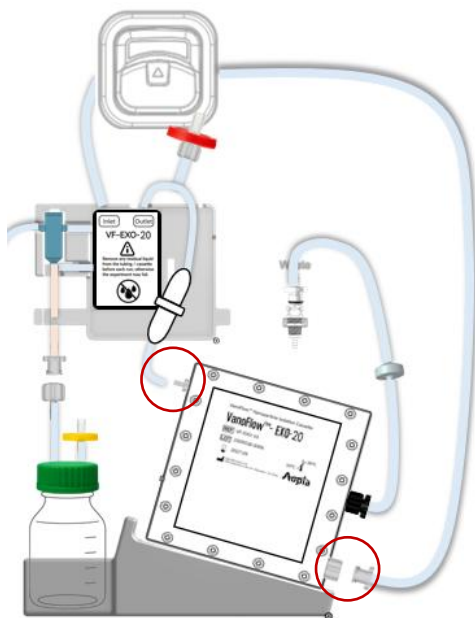
5.3 Step 3 - Post-Processing Procedures

5.3.1 Tubing Removal

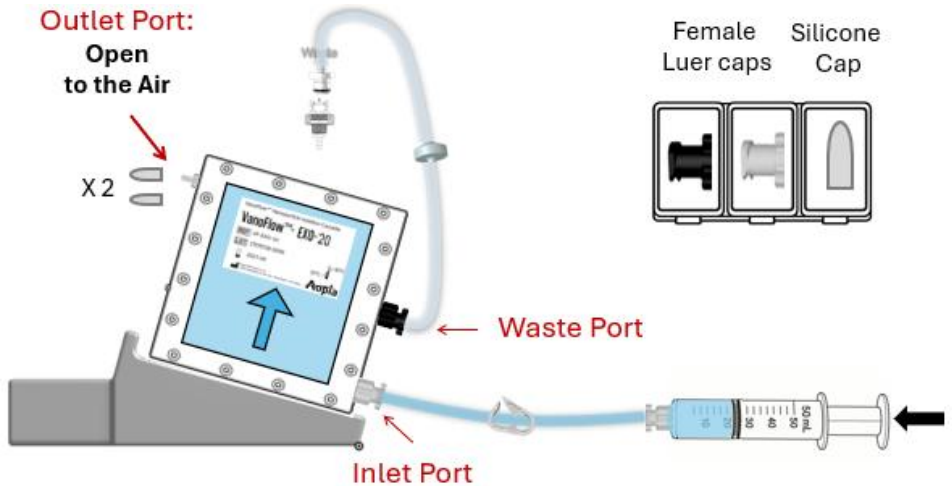
1. After confirming that the screen displays **“Collection Completed,”** the **“Release Cassette”** button at the bottom of the screen will become active (change from grey to green).

Release
Cassette

2. Click the **“Release Cassette”** button. All valves will open automatically. The tubing may now be disconnected from the cassette **as indicated by Red Circles.**
3. **DO NOT** disconnect the **Cassette Waste Tubing** if the cassette will be stored for reuse. Disconnect tubing only at the locations indicated by the red circles.



5.3.2 Cassette Storage for Re-use



The cassette may be stored for reuse by following the procedure below.

Notes:

- If the cassette will be reused within **48 hours**, the storage buffer loading procedure **may be skipped**. In this case, cap the inlet, outlet, and waste ports and store the cassette at **4–8 °C**, then reuse within 48 hours.

Storage Buffer Loading Procedure

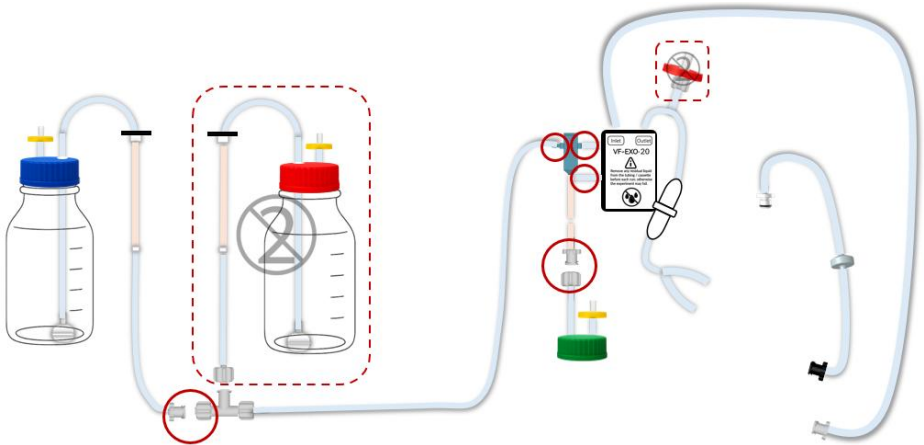
- **Required Materials (Accessory Kit):**
 - 50 mL Luer-lock syringe
 - Tubing with female Luer fittings on both ends
- **Storage Buffer:**
Cassette Storage Buffer (0.05 N HCl, **not provided**)
- **Procedure**
 1. Assemble the syringe and tubing, then draw **40 mL** of Cassette Storage Buffer into the syringe.
 2. Connect the syringe–tubing assembly to the **cassette inlet port** via the Luer-lock fitting (as shown above).

3. Verify that:
 - Ensure that the cassette is placed on the **cassette stand**
 - The **two (2) outlet ports (top left)** is open to air
 - The **cassette waste tubing remains connected** to the waste port.
 4. Slowly inject the storage buffer into the cassette until the internal chamber is completely filled.
(The buffer will reach the outlet port when the cassette is full.)
 5. Cap the **two (2) outlet ports** with the provided **silicone caps**.
 6. Continue gently injecting the storage buffer until **resistance is felt**.
 7. Remove the syringe–tubing assembly from the inlet port.
Note: Do not remove the waste tubing at this step, as this may cause buffer leakage from the inlet port.
 8. Cap the **inlet port** with the **transparent female Luer cap**.
 9. Remove the waste tubing and cap the **waste port** with the **black female Luer cap**.
- **Storage Conditions**
Store the cassette at **2–8 °C** for up to **4 weeks** before cleaning and reuse.

Cassette Storage Summary

Maximum reuse	Up to 2 reuses (total 3 processing cycles per cassette)
Short-term storage (no storage buffer)	Reuse within 48 hours at 4–8 °C
Long-term storage (with storage buffer)	Store at 2–8 °C for up to 4 weeks before cleaning and reuse
Freezing	Do not freeze the cassette

5.3.3 Cleaning and Storage of Reusable Tubing Set Components



General Instructions

- Clean all **reusable components** of the Tubing Set according to the procedure below, then store them for future use.
- Discard the following **single-use components** after each run:
 - Sample Feed Cap Assembly (**red cap**)
 - Venting Valve (**red filter**)
- Disassemble the remaining tubing set into individual components by disconnecting them **only at the locations indicated by the red circles** in the schematic above.

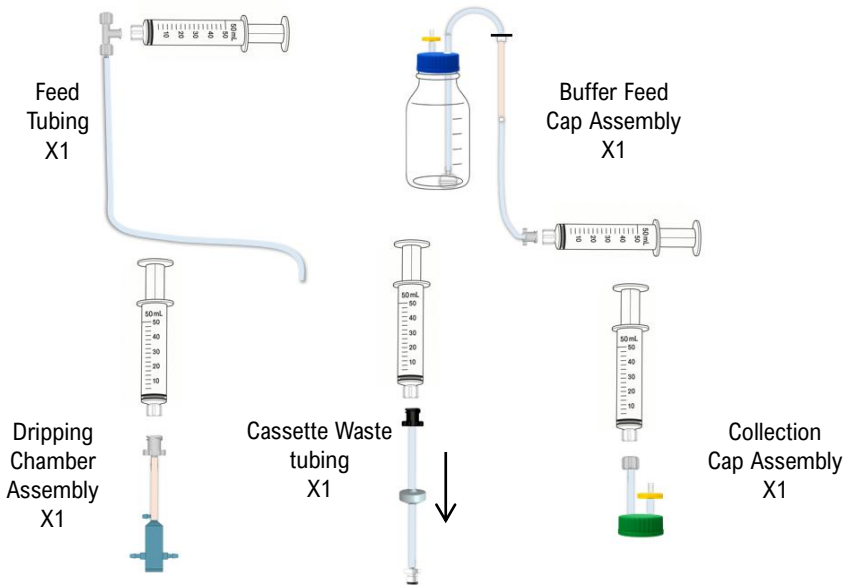
A. Cleaning of Reusable Tubing Components (except for Tubing hub)

Components requiring cleaning

- Feed Tubing
- Buffer Feed Cap Assembly
- Dripping Chamber Assembly
- Collection Cap Assembly
- Cassette Waste Tubing

Procedure

1. Using a **clean syringe**, flush each reusable tubing component thoroughly with **DI water**.



2. Flush each component with **70–75% ethanol or isopropyl alcohol (IPA)**.
3. Flush again with **DI water** to remove any residual ethanol or IPA.
4. Immediately **purge each component with air** using the syringe until minimal amount of liquid is present.



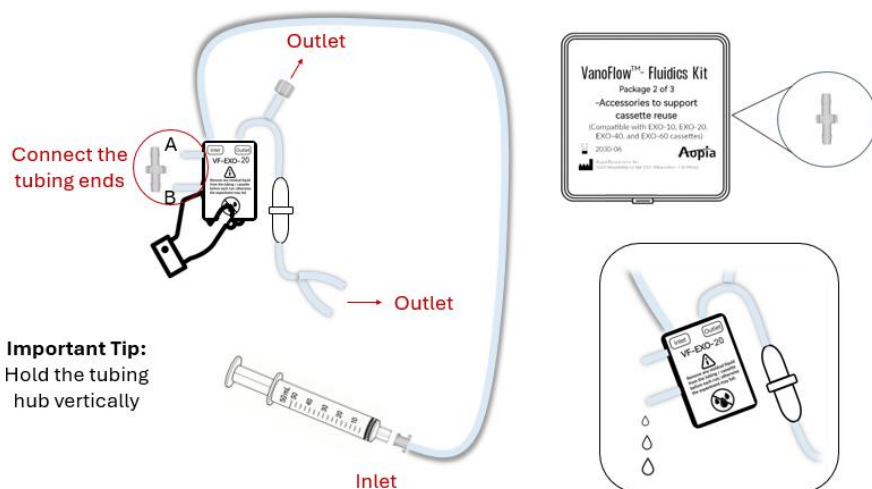
IMPORTANT

- Clean the **Cassette Waste Tubing** in the **allowed flow direction only**, as a check valve is present.
- The **yellow filters** on the **Buffer Feed Cap Assembly**, and **Collection Cap Assembly** are for **venting only**. **Do not wet or flush the yellow filters**, as this may cause failure during subsequent processing.

B. Cleaning of Tubing Hub (Cassette Inlet/Outlet Tubing Assembly)

Procedure

Tubing Hub (Cassette Inlet/outlet Tubing Assembly)



Important Tip:
Hold the tubing
hub vertically

1. Use the **barbed connector** (red circle, provided in the Accessory Kit) to connect tubing ends **A** and **B**.
2. Hold the **Tubing Hub (Cassette Inlet/Outlet Tubing Assembly)** **vertically** and use a **clean syringe** to flush the assembly from the **inlet side** with **DI water**, allowing the water to **exit through the outlet**.
3. Flush again with **70–75% ethanol or IPA**.
4. Flush again with **DI water** to remove residual ethanol or IPA.
5. Keeping the assembly **vertical**, empty the tubing by **pulling back the syringe plunger** to draw air through the outlet until no liquid enters the syringe.



IMPORTANT

- Pushing air into the tubing **cannot fully empty the assembly**.
- Air must be **drawn through** the tubing to remove residual liquid.

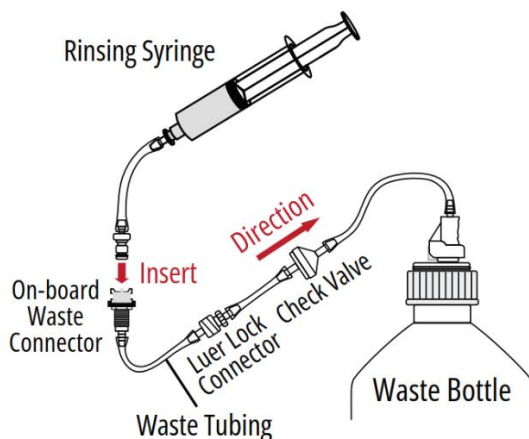
6. Remove the **barbed connector**.
7. Tilt the tubing hub until **no further liquid drips** from tubing end B, as shown in the schematic above.
8. Wipe exterior surfaces with **70–75% ethanol or IPA**.

C. Drying and Storage :

1. Wipe all cleaned components dry using a clean, lint-free absorbent pad.
2. Place the components in a **zip-lock bag** and store at **4–8 °C**.

5.3.4 Device Waste Line Cleaning

1. Using the **rinsing syringe** (provided in the smaller accessory box), flush the **waste bottle tubing** through the **waste bottle connector** with **20 mL distilled water**, followed by **20 mL of 70–75% isopropyl alcohol or ethanol**.
2. Immediately **push air through the tubing** to remove residual liquid and reduce the risk of microbial growth.



5.3.5 Exporting Run Logs (Optional)

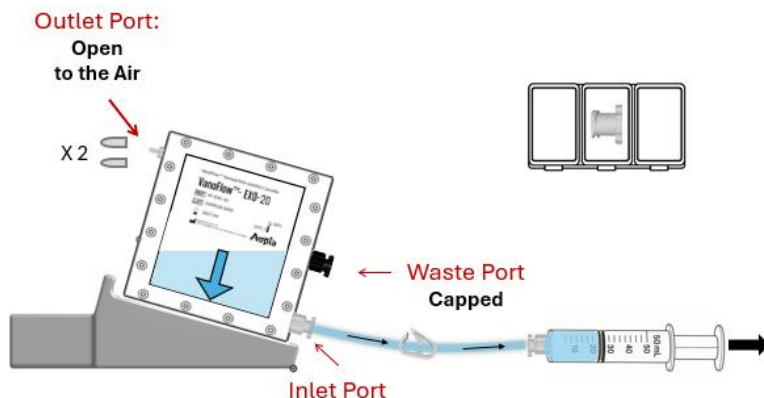
The NanoEX system generates a **run log (batch record)** for each completed run. The run log includes operation timestamps, software and protocol versions, cassette information, process parameters, equipment status checks, and key process events.

To export a run log:

1. Select **Log** on the Home screen.
2. Select the run record to export.
3. Insert a **USB flash drive** into the instrument.
4. Select **Export** to save the run log to the USB drive.

6. Processing Procedure – Reused Cassette

6.1 Step 1 - Cassette Cleaning



! IMPORTANT:

Cassette cleaning should be performed **within 48 hours prior to the next reuse** to ensure the membrane surface is freshly cleaned and ready for optimal performance.

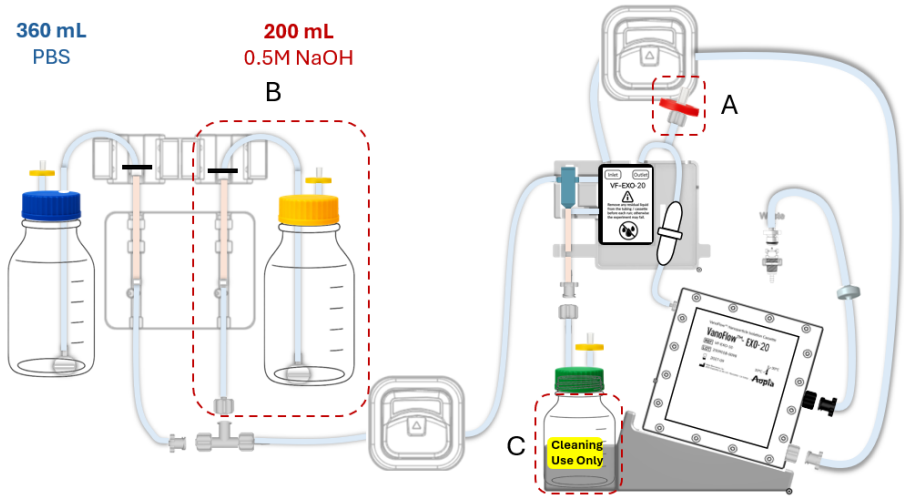
A. Remove Storage Buffer

1. Before cleaning and reusing the cassette, fully remove the **Cassette Storage Buffer**.
Note: Not removing or having too much residual storage buffer will prevent the automated cleaning process from proceeding.
2. Remove the **female Luer cap** from the **bottom inlet port** of the cassette and connect the **50 mL syringe-and-tubing assembly** to the inlet port (see *red circle* in the diagram above).
3. Remove **ALL** the **silicone caps (X2)** from the **top-left outlet ports** while keeping the **waste port capped**.

! IMPORTANT

- Do **not** remove the waste port cap at this stage.
- Removing the waste port cap may create negative pressure in the chamber and cause **membrane damage**.

- Using the syringe, withdraw the storage buffer until the cassette is completely empty.



B. Cassette Setup for Automated Cleaning

- Before each run using a reused cassette, an **automated cleaning protocol must be performed**.
- Follow the same procedure described in **Step 1 – Cassette Assembly** under **Processing Procedure – First Time Use**, with exceptions in the following steps:



IMPORTANT:

- Before proceeding, perform the tubing hub liquid removal procedure described section **5.1.5a Empty the Tubing Hub**.
- Residual liquid in the tubing hub **may interfere with automated operation**.

- Use a **Cleaning NFC Tag (VF-CLN-20)** instead of a Purification NFC Tag (VF-EXO-20).
- Open the **Cleaning Bag** and install the **Yellow Cap Cleaning Feed Cap Assembly**.
Use a **dedicated bottle for NaOH** (not provided).

5. Install a **new red venting valve** provided in the Cleaning Bag (see *red dashed box A* above).
6. Place the **NaOH Cleaning Bottle** on the **right side (sample side)** (see *red dashed box B* above).
When prompted, enter the **actual NaOH volume** in the sample volume field.
7. Prepare the cleaning solutions:
 - **200 mL of 0.5 M NaOH** in the Cleaning Bottle
 - **360 mL of PBS or Tris buffer** in the Buffer Bottle
8. Install the **reusable 100 mL NaOH Cleaning Collection Bottle** (yellow cap) provided in the NanoEX Accessory Kit onto the Collection Cap Assembly (see *red dashed box C* above).

C. Automated Cleaning

1. After completing cassette assembly, select “**Cleaning Mode**” on the Select Processing Mode page.
2. Follow the on-screen instructions to begin the **automated cleaning cycle**.
3. After cleaning is completed, perform a **Collection** cycle.
This step collects any remaining solution and fully empties the cassette and tubing and must be completed before starting the next purification run.
4. Discard the collected cleaning solution and clean the **NaOH Cleaning Collection Bottle and Yellow Cap Cleaning Feed Cap Assembly** for future use.



Important Notes

- NaOH effectively removes **protein- and lipid-based fouling**, but may be insufficient for other fouling mechanisms, e.g. cellulose from plant extract.
- If severe membrane fouling occurs, indicated by high processing pressure, up to 2 times the recommended NaOH volume may be used for cleaning. Do not use more than 2 times the recommended NaOH volume, as excessive NaOH exposure may cause **changes in membrane cutoff characteristics and pore enlargement**.

6.2 Step 2 – Purification

1. Open the **Reuse Bag** and install the following new components:
 - **Sample Feed Cap Assembly (red)**
 - **Red Venting Valve**
 - **New Collection Bottle with Cap**
2. Perform purification by following the procedures described in ***Processing Procedure – First Time Use.***



IMPORTANT:

Before proceeding, ensure that the tubing hub liquid removal steps described on section **5.1.5a Empty the Tubing Hub** have been completed.

7. Troubleshooting

Set up		
Problem	Possible Causes	Solution
Cassette not detected by the instrument (“Not detected”)	Malfunction of NFC reader	<ul style="list-style-type: none"> Restart the system
	NFC tag is damaged or not properly placed in the tubing adapter	<ul style="list-style-type: none"> Ensure the tubing adapter is properly installed and a new NFC tag is inserted
Cassette not recognized by the instrument (“Unknown”)	Software and Protocol Profile(.vf) are not up to date or NFC tag malfunction	<ul style="list-style-type: none"> Download and update latest software and Protocol Profile from: https://aopiabio.com/software If issue persists, try a new NFC tag
Process		
Problem	Possible Causes	Solution
Sample not detected by the sensor (Warning)	No sample/buffer added to bottle	<ul style="list-style-type: none"> Add sample/buffer to the sample bottle
	Suction ball not touching the bottom of the bottle	<ul style="list-style-type: none"> Ensure the suction ball is seated at the bottom of the bottle
Or	Tubing not inserted into sensor	<ul style="list-style-type: none"> Insert the tubing fully into the sensor
Buffer not detected by the sensor (Warning)	Feed tubing not properly loaded in pump	<ul style="list-style-type: none"> Open pump head, confirm tubing is positioned correctly, then close
	Pump head not closed	<ul style="list-style-type: none"> Close the pump head securely
	Incorrect Sample/Buffer Feed Cap Assembly used	<ul style="list-style-type: none"> Use the correct Sample/Buffer Feed Cap Assembly for this cassette model
	Luer Lock connection not tightened or leaking	<ul style="list-style-type: none"> Tighten Luer Lock connection; if issue persists, replace Sample/Buffer Feed Cap Assembly
Liquid is not detected in cassette (warning) Scenario 1: Liquid is present in dripping chamber ($\geq \frac{1}{4}$ height)	Dripping Chamber not seated against liquid sensors	<ul style="list-style-type: none"> Firmly press the Dripping Chamber into the sensors behind the adapter; when liquid is detected, red LED turns off Click Retry to continue
	Air bubble trapped in the dripping chamber or the tubing hub	<ul style="list-style-type: none"> Remove the bubble by lightly tapping the dripping chamber and tubing hub; when liquid is detected, red LED turns off Click Retry to continue

Liquid is not detected in cassette (warning) Scenario 2: liquid level < ¼ height of dripping chamber or chamber empty	Sample / buffer not sufficient or liquid not reaching sensor	<ul style="list-style-type: none"> • Abort experiment → Collect to remove remaining liquid in the cassette. • [Important] Empty the dripping chamber and tubing hub. Refer to section 5.1.5a Empty the Tubing Hub • Restart process and add buffer to the recommended volume
	Pinch valve not clamping tubing properly, liquid in the dripping chamber leaked.	
Tangential flow is not detected (warning)	Cassette circulation tubing not properly loaded in the tangential flow pump	• Ensure the cassette circulation tubing is correctly loaded in the Tangential Flow Pump
	Pump head not closed	• Close the pump head securely
Pre-test failed, unable to proceed (warning)	System detected an installation or priming issue	<ul style="list-style-type: none"> • Abort the experiment → go to Collect → collect all liquid from the cassette • Re-add buffer to the Buffer Bottle to the recommended volume • Before restarting, verify all installation points and ensure the correct NFC tag inserted
Sample was not completely loaded	Suction ball is not sitting at the bottom of the sample bottle	<ul style="list-style-type: none"> • Ensure the suction ball is fully seated at the bottom of the sample bottle so all liquid can be drawn • Re-position tubing if necessary
	Cassette pressure threshold was reached and the system automatically switched to washing	<ul style="list-style-type: none"> • Ensure the sample volume is within the cassette's rated capacity • Reduce sample volume if sample has high macromolecule or particle content • Dilute samples with buffer
Buffer was not completely loaded into the cassette	Suction ball is not sitting at the bottom of the buffer bottle	<ul style="list-style-type: none"> • Ensure the suction ball is fully seated at the bottom of the buffer bottle • Adjust tubing position if necessary
Cassette is filled with excessive air and bubbles	Incorrect sample volume input, causing the sample to run out before the system switched to buffer.	• Ensure the entered sample volume does not deviate from the actual sample volume by more than ±10%
	Unexpected sample loss (spillage, sample removal)	• Confirm the actual volume in the bottle is equal to or greater than the entered volume before starting.

The sensor LED is blinking	The power supply may be unstable or experiencing a surge.	<ul style="list-style-type: none"> • Connect the instrument to a stable / regulated power source.
Collect		
Problem	Possible Causes	Solution
Elution volume too high	Buffer bottle was not empty during the collection step	<ul style="list-style-type: none"> • Empty the buffer bottle before starting collection
Elution volume is too low	Air entered the system before collection due to actual sample volume significantly less than the input volume number.	<ul style="list-style-type: none"> • Verify sample volume entry is accurate
	Purified samples are still trapped in the dripping chamber and tubing hub	<ul style="list-style-type: none"> • Empty the dripping chamber and tubing hub to recover all the purified samples. Refer to section 5.1.5a Empty the Tubing Hub
Performance		
Problem	Possible Causes	Solution
Low Purity	Sample volume exceeded cassette rated capacity	<ul style="list-style-type: none"> • Ensure the sample volume is within the rated capacity of the cassette
	Insufficient buffer loaded for proper washing	<ul style="list-style-type: none"> • Ensure adequate buffer is loaded according to the recommended volume
	Highly complex sample matrix	<ul style="list-style-type: none"> • Reduce the sample volume and consider diluting the sample before loading; optimize the sample preparation step as needed.
Low Yield	Non-specific adsorption or sample aggregation	<ul style="list-style-type: none"> • Use fresh sample and select the appropriate cassette size
Software Update		
Problem	Possible Causes	Solution
USB drive is not detected	USB not fully inserted or not formatted correctly	<ul style="list-style-type: none"> • Re-insert the USB drive (the one provided with the instrument is preferred). • Try another USB port or use a different USB drive.
After updating, NFC is still not recognized	System has not refreshed NFC driver	<ul style="list-style-type: none"> • Restart the system and retry • Ensure the correct Protocol Profile is installed

8. Additional Information

Material Compatibility

Use only recommended buffers and solutions. If other reagents are used, first check chemical compatibility for the following materials used in the cassette:

- Polypropylene (PP)
- Acrylic (PMMA)
- Silicone tubing
- PVC tubing
- Polycarbonate (PC)

Appendix 1: Sample Cooling Guide (Example Method)

Purpose

- This guide provides an **example method** for maintaining a low sample and buffer temperature during processing using a **simple, passive cooling approach**.
- This information is for reference only and is not intended to define or guarantee system performance.

Example Cooling Setup

- Place the **sample bottle** and **buffer bottle** in an **insulated container**, e.g. a Styrofoam box.
- Position **cold gel packs** around the bottles to provide passive cooling.
- The cold gel packs should **fill the remaining empty space** within the insulated container to provide sufficient cooling capacity.
- Ensure that bottles remain upright and stable throughout the run.
- **A lid is not required**, provided the container offers sufficient thermal insulation.

Example Configurations Evaluated

Two example configurations were evaluated to illustrate cooling performance for **different bottle volumes**.

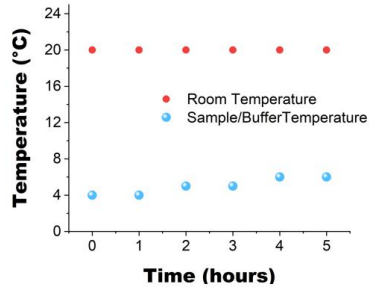
These examples are provided to demonstrate **practical setups**, not to establish requirements.

Example	Bottle Volume	Insulated Container (Internal Dimensions)	Cold Gel Packs	Initial Tem.
Example A	500 mL bottles	Small insulated box: approx. 24 x 14 x 15 cm (L x W x H)	4x gel packs (approx. 250 ml each)	~4 °C
Example B	2 L bottles	Large insulated box: approx. 29 x 23 x 23 cm (L x W x H)	8x gel packs (approx. 250 ml each)	~4 °C

Example A – 500 mL bottles



Example B – 2 L bottles



Example Performance Observation

In internal testing using the example setup described above:

- The sample and buffer bottles were placed in an insulated container with ice packs.
- Over a **5-hour period**, the observed temperature increase was approximately **2 °C** as shown in the figure above (from 4 to 6 °C). (Room Temperature = 20 °C)
- Actual temperature performance may vary depending on insulation quality, ice pack size, ambient temperature, and bottle volume.

Summary

Using an insulated container with cold gel packs is a simple and practical method to limit temperature increase during processing, particularly for temperature-sensitive samples such as culture media.

Appendix 2: VanoFlow-EXO-20 Cassette Quick Guide

Continue to Next Page



VanoFlow[®]-EXO-20 EV Isolation Cassette Quick Guide

Doc Num: QG-VF-EXO-20 Rev A



Materials

Materials provided	Qty
VanoFlow-EXO-20 cassette	1
VanoFlow-EXO-20 Tubing set bag (First Use, 2x Reuse, Cleaning)	1
Sample collection bottle (100mL)	3
NFC tags (3x Purification + 3x Cleaning)	6
Part storage box	2

Materials Required but Not Provided (Each Run)	Qty
0.05M HCl (for storage)	40 mL
0.5M NaOH (for cleaning)	200 mL
PBS or TBS buffer	360 mL
70–75% Ethanol or Isopropyl alcohol	200 mL
DI water or distill water	200 mL
GL-45 bottles (Sample, Buffer, and Cleaning)	3
Zip lock bag (for tubing storage)	1

Software Requirement

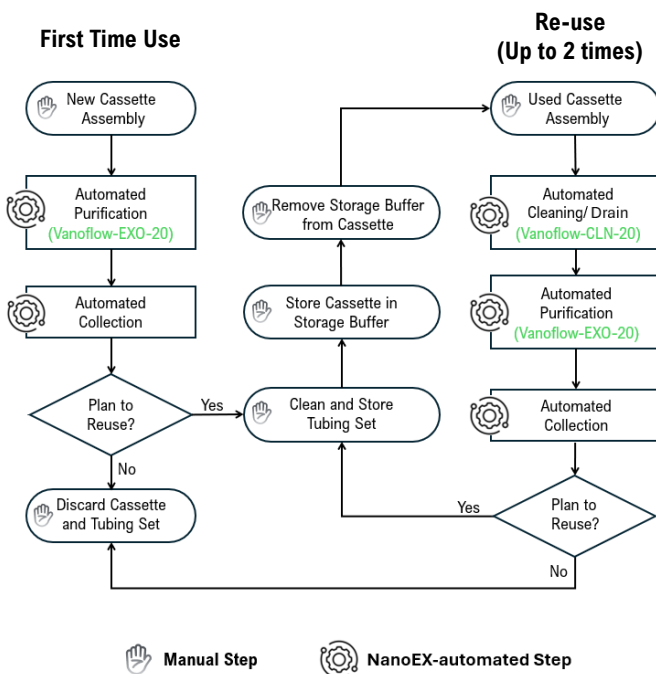
- Version **V3.5.4** or later for instrument with serial number (last 4 digit) \leq 0030
- Version **V5.5.4** or later for instrument with serial number (last 4 digit) \geq 0031
- Protocol Profile **V2.6.1** or later

Safety

WARNING: Sodium hydroxide (NaOH) and hydrochloric acid (HCl) solutions are **highly corrosive** and may cause **severe skin burns, eye damage, and respiratory irritation** if mishandled.

- Use appropriate Personal Protective Equipment (PPE)
- Dispose of waste according to local and government regulations.

Workflow Overview



First Time Use

Cassette Assembly

- Insert “**Purification**” tag (VF-EXO-20) into the rear slot of the **Tubing Adapter** and verify that the correct cassette name is displayed on the instrument screen.
- Install **Sample** and **Buffer Bottles** (not provided) and connect the associated tubing.
- Place **Cassette** and **Collection Bottle** (provided) onto the **Cassette Stand**.
- Press the **Waste Connector** thumb latch, insert the **Cassette Waste Tubing** into the connector, and place the waste container at the same level as the instrument.
- Connect all tubing as indicated by the **Red Circles** in Figure 1.
- Install the tubing onto the **pump heads**.

Reminder: When reusing a process tubing, confirm that the **tubing hub emptying** steps in Figure 6 are complete.

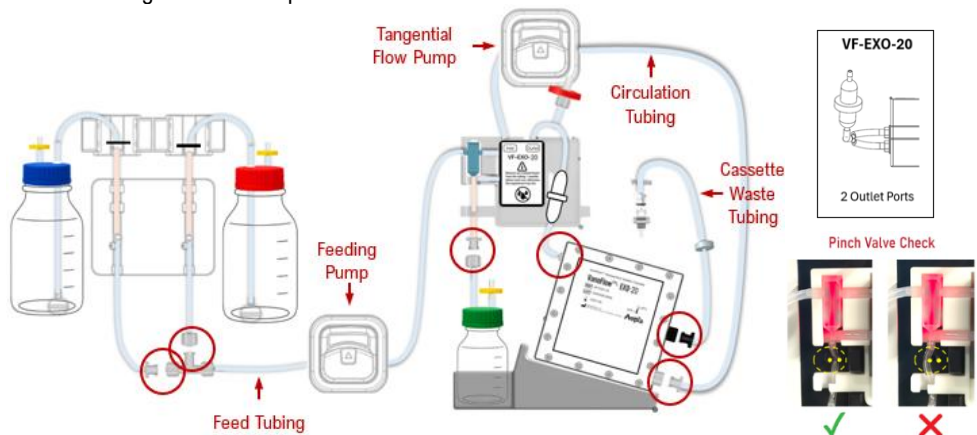



Figure 1 - Tubing connection diagram

Automated Processing


- On the **Home** screen, click **Set Up**, then select the processing mode. The “**Standard Mode**” is always recommended for most sample types.
- Follow the on-screen instructions to complete the required checkpoints. **Verify** that the pinch valve has **securely clamped** the tubing, as shown in Figure 1 (right).
- When prompted, input sample name and volume.
- Add **sample** and **buffer (360 mL)** to the corresponding bottles.
- On the main processing screen, press the () button to begin processing.

Reminder:

-The system performs automated pre-checks during the initial **~12 minutes** before sample loading.

-Monitor the system during this period. After sample loading begins, the run typically proceeds without further user intervention.

Collection

- After processing is completed, click the “**OK**” button on the pop-up window to return to home screen.
- On the Home screen, click “**Collect**” and follow the on-screen instructions.
- Before starting collection, cap the **venting valve** with the provided **silicone cap**.
- On the main processing screen, press the () button to begin collection.

Post-processing Procedures

- After collection is completed, click the **“Release Cassette”** button (now activated).
- If planning to reuse the cassette, disconnect only the tubing indicated by **Red Circles** in Figure 2(left) and keep the other tubing connected.
- Using a **50mL syringe**, slowly inject **~40ml storage buffer** (0.05M HCl) into the cassette as shown in Figure 2(right). When the buffer reaches the Outlet Port, cap the **Outlet Ports** (x2) with silicone caps (x2).
- Continue injecting more storage buffer into the cassette until resistance is felt. Remove syringe and cap the **Inlet Port** with a Clear Luer Cap, then disconnect the waste tubing and cap the **Waste Port** with a Black Luer Cap. Store at 2-8°C for up to 4 weeks.
- Flush the **Waste Bottle Tubing** through the Waste Bottle Connector using the Rinsing Syringe (provided) with 20mL Distilled H₂O followed by 20ml 70%-75% Isopropyl alcohol or Ethanol and then push air through the tubing.

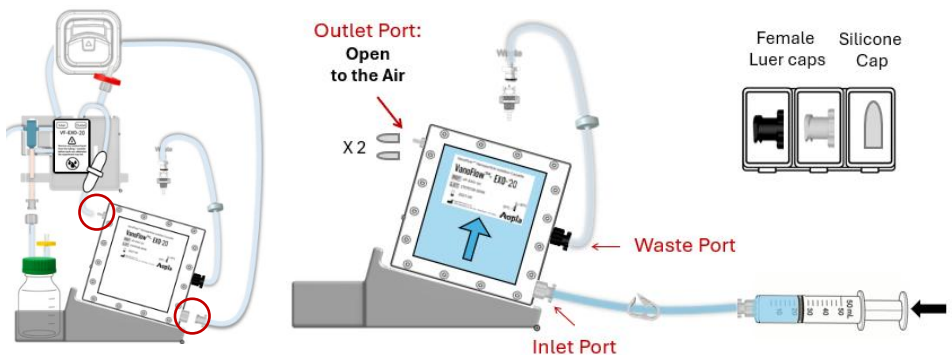


Figure 2 - Cassette storage for future use

- Discard the used **Sample Feed Cap Assembly (red cap)** and the **Venting Valve (red filter)**.
- Disassemble the remaining tubing set into individual components by disconnecting them as indicated by the **Red Circles** in Figure 3.

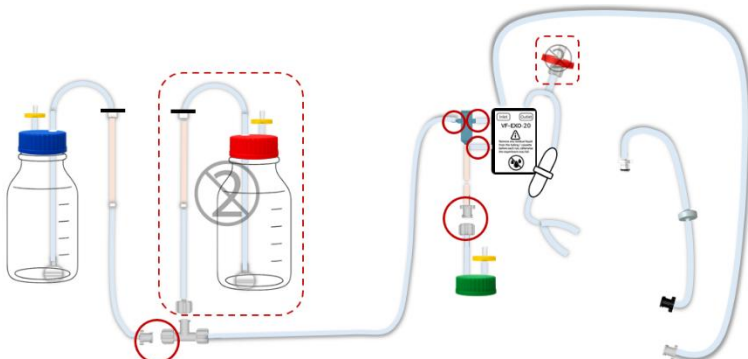


Figure 3 - Disassembling tubing for cleaning

Tubing Cleaning for Reuse

- **A. Standard Tubing (excluding Tubing Hub):** Flush each tubing with DI water, followed by 70–75% Ethanol (or IPA), and flush again with DI water. Purge with air to remove residual liquid.
- **B. Tubing Hub:** Clean the **Tubing Hub** (Cassette Inlet/Outlet Tubing Assembly) according to Figure 4.
 - Connect tubing ends with the **Barbed Connector** (provided) indicated by **Red Circle**.
 - Hold the tubing hub **vertically** and flush from the inlet side with DI water, follow by 70–75% Ethanol (or IPA), and flush again with DI water. Keep the tubing hub vertical and **draw air by pulling back the syringe plunger to empty**.
 - **IMPORTANT:** Do not push air through the tubing hub. Residual liquid may remain.
 - Remove the **Barbed Connector** and tilt the **Tubing Hub** to remove residual liquid.
- Air dry all the reusable components and store in a zip lock bag at 2–8°C until reused.

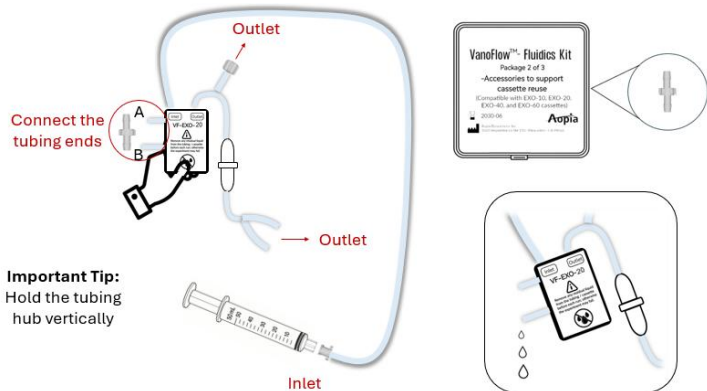


Figure 4 - Cassette inlet/outlet tubing assembly cleaning

Reusing a Cassette

Removing Cassette Storage Buffer

- Remove **All** the silicone caps (x2) from the cassette while keeping the Waste Port capped. Connect a syringe to the Inlet Port and draw the cassette storage buffer from the cassette, as shown in Figure 5.

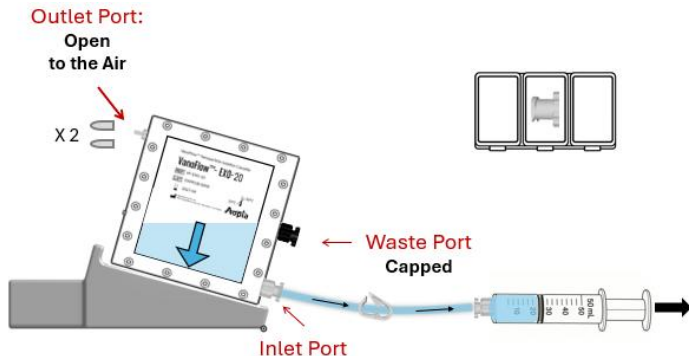


Figure 5 - Storage buffer removal

Emptying Tubing Hub

- Use the pump head to pinch (close) **the inlet tubing**.
IMPORTANT: This step is **required** before proceeding.
- Hold the tubing hub **vertically** during aspiration to facilitate complete liquid removal.
- Using a pipette with a **1 mL tip**, **fully insert the tip into tubing ends A and B to form a seal**, then aspirate any remaining liquid from tubing ends A and B **until no liquid can be withdrawn**, as shown in Figure 6.
- Replace the venting valve with a **new, venting valve**.

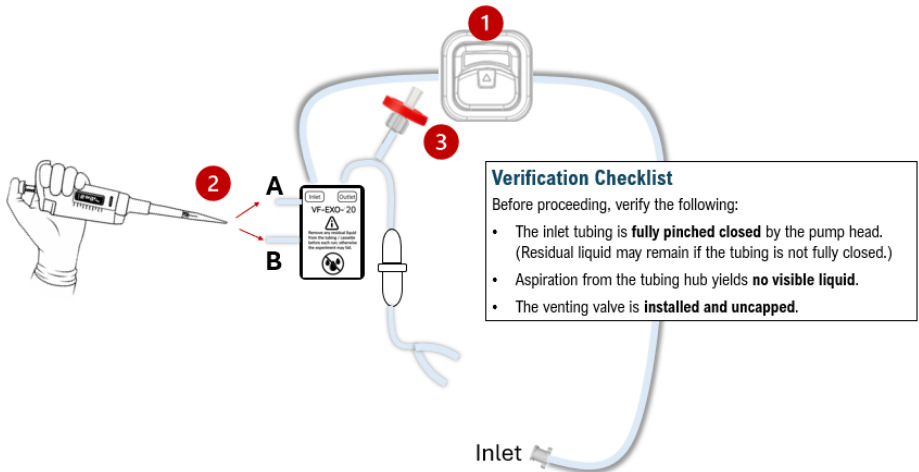


Figure 6 – Emptying tubing hub

Cassette Cleaning

- Assemble the reused cassette and tubing following the same procedure as **First Time Use -> Cassette Assembly**. However, use a “**Cleaning**” NFC Tag (VF-CLN-20) instead of “Purification” Tag (VF-EXO-20).
- Before automated cleaning, confirm that the **tubing hub emptying** steps in Figure 6 are complete.
- Ensure a new **Red Venting Valve** is installed in the process tubing (provided), as shown in Figure 7A.
- Connect the **Yellow Cap Cleaning Feed Cap Assembly** (provided) to a dedicated GL-45 cleaning bottle for NaOH (not provided), as shown in Figure 7B.
- Fill the bottles as follows:
 - **200mL** of 0.5M NaOH in the Cleaning Bottle (Right)
 - **360mL** of PBS or Tris buffer (Do not use non-buffering solution) in the Buffer Bottle (Left).
- Install the reusable 100mL **Cleaning Collection Bottle** (provided) onto the **Collection Cap Assembly** to collect the cleaning solution, as shown in Figure 7C.
- Select “**Cleaning Mode**” on the “Select Processing Mode” page. Follow screen instructions to perform automated NaOH clean.
IMPORTANT: After the cleaning process is completed, perform a Collection cycle.

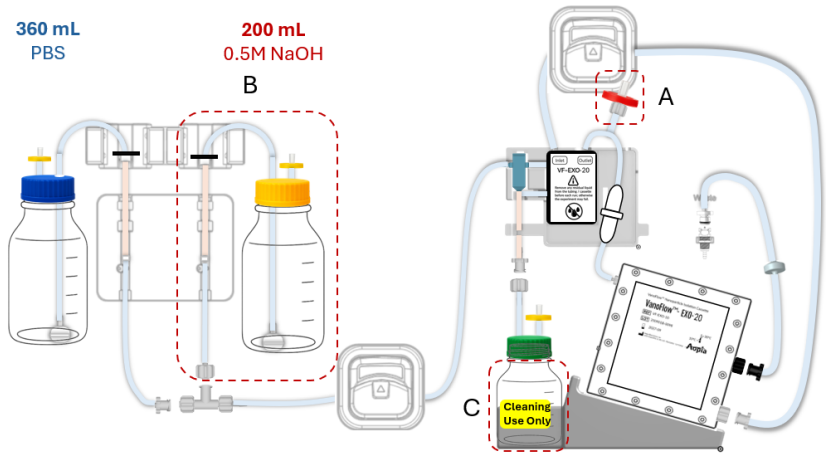


Figure 7 - Set up for automated NaOH cleaning

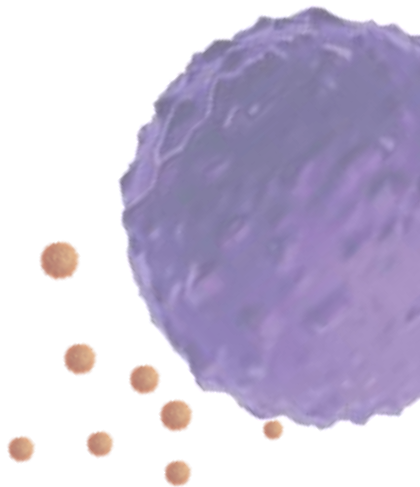
Sample Processing

- After the cleaning cycle is completed, perform purification by following the procedures in **First Time Use** → **Begin Processing**.
- Before beginning Processing, confirm that the **tubing hub emptying** steps in Figure 6 are complete.
- Ensure to use a new set of **Sample Feed (red) Cap Assembly, Red Venting Valve, and Collection Bottle with cap**.

Common Installation Errors and Consequences

Common Installation Error	Consequence
Failure to empty the Tubing Hub before each run, as described in Figure 6, " Emptying the Tubing Hub. "	The pre-test may fail, and sample loading may be incomplete or result in excessive elution volume.
Failure to replace the red venting valve with a new one before each run.	
Tubing is not properly positioned between the two pins of the pinch valve, resulting in incomplete pinching.	The processing will fail
Failure to cap the venting valve during collection .	Incomplete sample collection

For the complete User Manual, scan the QR code below.



Aopia Biosciences, Inc.
1020 Serpentine Ln Ste 110, Pleasanton, CA 94566
www.aopiabio.com
info@aopiabio.com