NanoAssemblr Blaze™ User Guide

Power to Scale

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The Blaze is research use only. Not for in human use.

Safety Warnings are indicated throughout the guide. Please read through the whole guide and take special note of safety warnings.

1 Introduction

As part of the NanoAssemblr platform, the NanoAssemblr Blaze enables the rapid and controlled formulation and scaling of nanomedicines. The NanoAssemblr Blaze is designed to formulate transformative nanomedicines at intermediate scale for late preclinical studies and process development activities, such as:

1. Selecting a Nanomedicine Candidate

   Candidate drugs must show potency and non-toxicity in larger scale animal studies. These important studies can be conducted efficiently with a process that mirrors a clinical scale implementation.

2. Identifying a Scalable Manufacturing Process

   Process development can be conducted on both the upstream and downstream steps, from material preparation to buffer exchange, filtering and analytics, to ensure that a drug program is ready to quickly accelerate to the clinic.

The NanoAssemblr Blaze with NxGen Technology positions you to accelerate the creation of transformative medicines. This guide will detail how anyone can use the instrument to produce nanoparticles successfully and repeatedly.

2 Specifications

The NanoAssemblr Blaze specifications are shown in the following table:

Area	Specifications
Electrical power supply	• Voltage: 100–240 VAC • Frequency: 50–60 Hz • Current: 1.2 A
Electrical input to instrument	• Voltage: 24 VDC • Current: 3.75 A
Environmental	• Temperature: 15–35 °C • Humidity: 30–65% (non-condensing) • Pressure: 70–106 kPa • Ingress protection: IP20
Dimensions	• With lid closed: 89.3 x 46.6 x 65.0 cm • With lid open: 89.3 x 51.1 x 122.1 cm • Weight: 127 kg **Important:** The minimum weight the lab bench must support is 200 kg.
Tolerances	• Flow rate accuracy: +/- 2.5% (average) • Formulation pressure: Up to 150 psi • Volume accuracy: -4% to +2%

Table 1: *Specifications (Subject to change. For the most current version, visit precisionnanosystems.com/our-technology/blaze)*

The formulation parameter ranges for the NanoAssemblr Blaze with NxGen Technology are listed in the table below:

Parameter	Allowable range
Volume	10–1000mL, up to 10 L with Blaze+™
Reagent ratio	1:1–5:1
Total flow rate	4–115 mL/min*
Flow rate range for Center and Right channels	2–86.5 mL/min
Flow rate range for Dilution channel	4–230 mL/min
Dilution ratio	1:1 up to 4:1*
Start, End Waste	0 mL up to total volume

Table 2: *Parameter Ranges (*some parameters are dependent on the cartridge type)*

3 Installation

To ensure proper operation, the Blaze system will be installed by a Precision Nanosystems representative.

Each instrument comes with the following accessories:

- Power Supply and Power Cable
- Laptop and USB Cable
- Input Vessel Holders
- Output Vessel Holders
- Spare set of Sippers
- Cleaning Cartridge
- 50, 250, 500 mL bottles

Figure 3-1: *Blaze Instrument with Accessories*

4 System Introduction

The Blaze system has been designed to reproducibly formulate nanoparticles using precise, automated fluid handling in combination with the NanoAssemblr microfluidic mixers. At its heart, the Blaze is designed to create the homogeneous conditions crucial to producing high quality nanoparticle formulations. These high-quality nanoparticle formulations can then be scaled seamlessly across the NanoAssemblr Platform.

The mixer technology is integrated into convenient single-use cartridges that form part of the Blaze fluid path. The Blaze dispenses fluid at precise flow rates and flow rate ratios using syringe pumps, which are controlled through the Blaze software installed on the accompanying laptop.

4.1 Microfluidic Cartridges

Two mixer geometries are available on the Blaze: Classic and NxGen. Both cartridge geometries combine reagents from the Center and Right channels under precisely controlled, non-turbulent conditions to produce homogeneous nanoparticles. The resulting formulation is then delivered to the collection vessels.

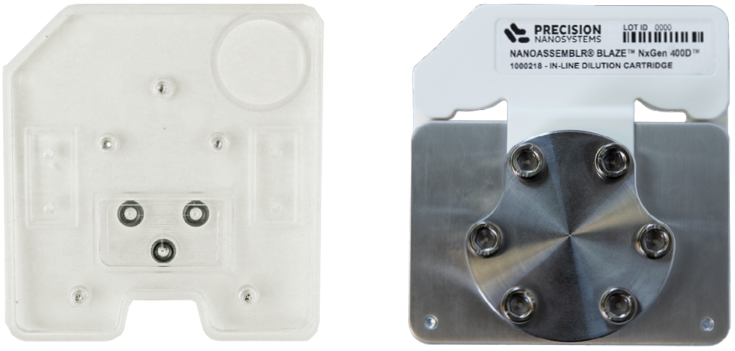


Figure 4-1: *Blaze Classic (left) and NxGen (right) Cartridges*

Both cartridge geometries are available with and without in-line dilution. Dilution cartridges mix the reagents from the Center and the Right channels in the same manner as non-dilution cartridges but have the added capability of introducing the dilution reagent at the output of the mixer. This enables precise in-line dilution of the formulation. See Section 4.1.1 In-line Dilution Considerations for more information.

KEY CONSIDERATIONS

It is critical to consider upstream and downstream processes during formulation development to determine if in-line dilution will be necessary.

Figure 4-2: *Blaze Classic (left) and NxGen (right) Cartridges with Dilution*

The following table lists the Volume and Flow Rate ranges that can be used for the two cartridge types.

Cartridge Type	**Pre-Dilution Volume (mL)**	**Flow Rate (mL/min)**		
		Center and Right		**Dilution**
		Total	Per Channel	
Classic	10 to 1000	4 to 18	2 to 15	4 to 72
NxGen	20 to 1000*	4 to 115	2 to 86	4 to 230

Table 3: *Blaze Cartridge Parameters *Up to 10 L with Blaze+*

KEY CONSIDERATION

The per channel flow rates can limit the Flow Rate Ratios at low and high Total Flow Rates i.e. at 115 mL/min Total Flow Rate, a Flow Rate Ratio of 3:1 is the maximum. A Flow Rate Ratio of 4:1 would result in one channel running at 93 mL/min, which is above the Center and Right Flow Rate limit.

A reusable Cleaning cartridge is provided with the Blaze for the purpose of running **Purge** and **Clean** Recipe Actions. The Cleaning cartridge is not capable of formulating.

4.1.1 In-line Dilution Considerations

Dilution of the formulation is desirable in some cases to reduce the proportion of the organic solvent in the output container. In-line dilution ensures all portions of the formulation are under the same dilution ratio immediately after mixing. The extent of dilution required, if at all, depends on the specific formulation and the intended downstream processes. Some factors that make in-line dilution desirable are outlined

below:

1. The formulation produced at the beginning of the run will rest in the output vessel for a longer period than the formulation produced near the end of the run. Depending on the duration of the process, the proportion of organic solvent in the output and the stability of the formulation in the solvent, this may lead to heterogeneous results for some formulations.

   While Blaze formulation may not allow enough time for such effects to be observed, longer formulations at later development stages may. Blaze allows testing of dilution conditions at smaller scales to mitigate risks for such heterogeneous effects at larger scales.

2. Depending on the formulation, some downstream processing such as tangential flow filtration (TFF) can subject the formulation to shear stress. In the presence of an organic solvent, such processing can lead to alteration of the particle size, size distribution, or morphology, affecting the performance of the formulation. Dilution is desirable to reduce the proportion of the organic solvent in the bulk formulation prior to TFF.

Stability of the formulation in the presence of solvent should be tested over a range of time to determine if long formulation runs and/or downstream processing pose a risk to the integrity of the formulation. As all formulations are unique, the parameters for in-line dilution must be optimized for the intended process.

4.1.2 NxGen Technology

NxGen technology is an innovative mixing architecture that is geometrically scalable to enable larger volumetric flow rates using the same mixing principle simplifying the scale-up of production across all stages of drug development through its ability to maintain precise, time-invariant mixing at flow rates ranging from millilitres per minute to litres per hour.

The Blaze system supports two NxGen cartridge variations, NxGen 400 and NxGen 500, along with dilution options (NxGen 400D and NxGen 500D). Both the NxGen 400 and NxGen 500 cartridges use the same architecture in different mixer dimensions. While both variations operate in the same time-invariant regime, they offer different flow and shear rates to better suit the nanoparticle formulation design space.

As a general guideline, NxGen 400 cartridges work well at total flow rates at or above 60 mL/min while NxGen 500 works well at total flow rates at or above 90 mL/min. These guidelines are highly dependent on formulation specifics and should be characterized independently.

NxGen 400 and NxGen 500 cartridges are also able to run at high throughputs for clinical development and commercial production on the NanoAssemblr GMP System. The NxGen cartridges used on Blaze are also used in the GMP System, further

mitigating scale-up risks.

4.2 System Components

The following images shows the physical components of the Blaze instrument. The component names are used throughout this guide. Refer to these images as needed.

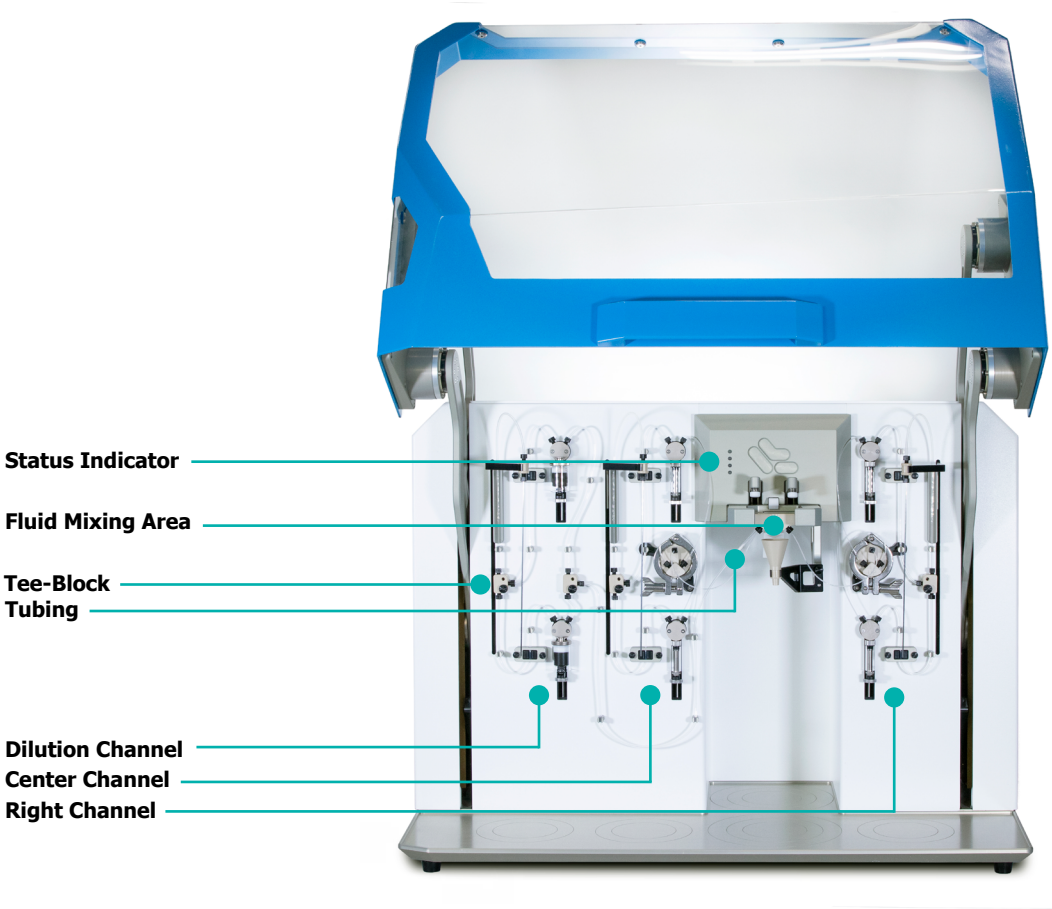


Figure 4-3: *Blaze Components*

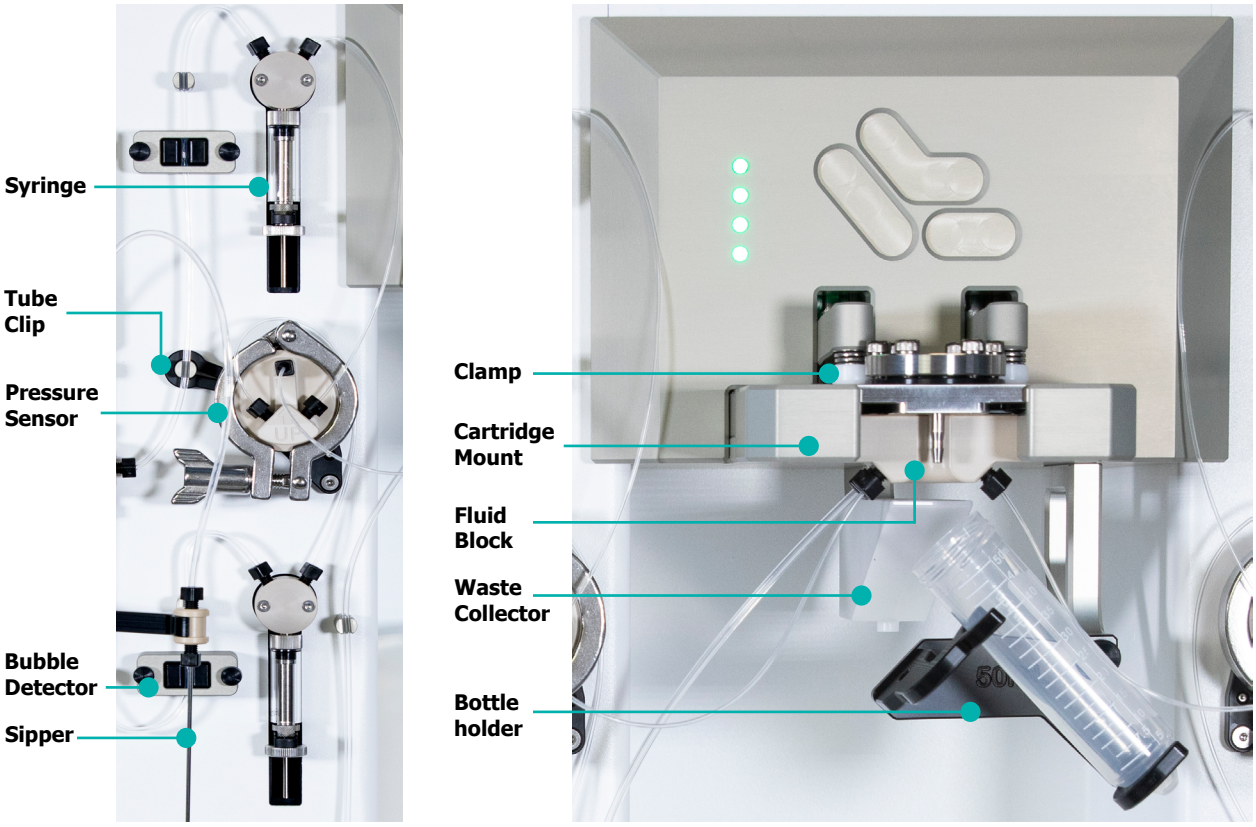


Figure 4-3: *Blaze Components Cont.*

4.2.1 Glossary of System Components

For this section refer to Figure 4-3. Select components requiring further explanation in this guide are defined below.

Tee-blocks: Tee-blocks combine or split two fluid streams.

Bubble Detectors: Bubble detectors can facilitate fluid path priming and bubble detection. The bubble detectors can detect bubbles in the fluid path during a formulation, and end the formulation based on selected sensitivity. See Section 10.2 Bubble Detection.

Pressure Sensors: The pressure sensors log the pressure in the fluid path during a formulation, providing a diagnostic tool and overpressure protection.

Tube Clips: Tube clips hold the tubing in the proper positions.

4.3 Fluid Path

Blaze has been designed to continuously move fluids from the input bottles, through the cartridge, and into the sample collection bottle. Input reagents flow through the fluid path using a variety of components (detailed in Figure 4-3 and Section 4.2 System Components) to ensure consistent and accurate flow characteristics.

The fluid path can be described as having three distinct channels converging at the cartridge. They are termed as the Dilution, Center and Right channels. A single channel's fluid path is shown and described below.

Figure 4-4: *Blaze Fluid Path*

1. Fluid enters the fluid path through the sippers, drawn up from the input bottles into flexible tubing.
2. The fluid is then drawn into one of two syringes. Both syringes work in vertically aligned pairs to achieve continuous flow. Bubble detectors are positioned between the sippers and the syringes to detect when fluid first enters the fluid path at the beginning of a formulation.
3. The syringes push the fluid towards the fluid block, passing through a pressure sensor in the Center and Right channels, providing a diagnostic tool and over-pressure protection.
4. The fluid enters the fluid block, which channels the fluid to the mixer in the cartridge.
5. As the fluid exits the cartridge, it either enters the waste collector or flows into the sample collection bottle.

The fluid path is not replaced for each formulation. It is important to clean the fluid path in between formulations and to flush the channels with suitable solvents prior to formulating.

KEY CONSIDERATIONS

The hold-up volume is the volume of the fluid path for an individual input channel. Each channel has a hold-up volume of approximately 5 mL. This must be included when preparing sufficient volume of input reagents to ensure the channels do not run dry during formulation.

4.4 Status Indicator Lights on Blaze Instrument

Status indicator lights show the general status of the Blaze. These lights provide instrument status while monitoring a run. The status buttons in the software can be used when preparing for a Formulation for more detail.

Color	Status
Green	The instrument is idle and ready to accept commands.
Red	The instrument has stopped unexpectedly. Either an error has occurred or an operation was interrupted by opening the lid, using the **Emergency Stop** button or, for certain operations using the respective **Stop** button.
Dark Blue	The instrument is performing a Recipe Action such as clamping, cleaning or formulating.
Yellow	An operation has been interrupted and moving components are being returned to their home positions. If this occurs during a formulation, consult the formulation results screen or the history to determine the cause of the interruption. See Section 11.1 History.
Purple	The instrument is seeking to connect with the computer.

4.5 Glossary of Terms and System Parameters

Flow Rate Ratio: The ratio of the Center and Right channel flow rates.

Dilution Ratio: The flow rate ratio of the Dilution and combined Center and Right channel flow rates.

Total Flow Rate: The total rate of fluid flow through the Blaze cartridge from the Center and Right channels. This is equal to the sum of the Center and Right channel flow rates.

Start Waste: The amount of fluid diverted to the waste container at the start of each formulation.

End Waste: The amount of fluid diverted to the waste container at the end of each formulation.

Hold up Volume: The amount of fluid remaining in the fluid path after a formulation.

5 Workflow

For general use and operation, the workflow is outlined below:

Step	Action
1.	**Starting the Blaze instrument** Powering up, connecting to the laptop, and initializing the system
2.	**Preparing the Instrument for a Formulation** Installing the cartridge, input and output vessels, and checking the status
3.	**Preparing the Fluid Path for a Formulation** Performing a sanitization or wash protocol, and flushing the fluid path
4.	**Formulating on Blaze** Creating recipes, running and monitoring the formulation
5.	**Post-Formulation** Performing a post-formulation clean, accessing the formulation history, and exporting recipe and history information

Recipe Actions used in this workflow are described below. A Recipe Action is a step or set of steps performed by the instrument in response to selecting a button on the Recipes page of the software. Recipe Actions include the following:

Clamp/Unclamp: Clamps or unclamps an installed Cartridge. Clamping ensures a tight seal between the Cartridge and the Fluid Block and is required for **Clean**, **Purge** and **Formulate** Recipe Actions.

Clean: Draws in cleaning reagents and pushes the fluid through the entire fluid path, including the cartridge, then into the waste container. A new, previously used or cleaning cartridge may be used.

Select a cleaning time and load the appropriate volume of reagents noted in the table on the following page.

Duration	Dilution	Center	Right
2 Min	50 mL	15 mL	15 mL
10 Min	250 mL	75 mL	75 mL
60 Min	1450 mL	450 mL	450 mL

Table 4: *Cleaning Durations and Corresponding Volumes*

Ten minutes is the recommended **Clean** duration — this Recipe Action flushes the fluid path with 10 times its hold-up volume.

Recover: Empties the fluid path by pumping the contents backwards and out through the sippers. Users can opt to keep the recovered fluid if desired. No cartridge should be loaded for this Recipe Action to avoid cross-contamination between the channels.

Duration	Description
30 seconds	Empties the fluid path
2 minutes	Empties the fluid path and continues to pump air through to dislodge stray droplets and dry the channels

Table 5: *Recover Durations and Corresponding Descriptions*

KEY CONSIDERATIONS

A **Recover** is the best way to remove fluid from the fluid path and should always precede a formulation.

Purge: Empties the fluid path by replacing the contents with air. The contents are pumped through the cartridge and into the waste container. A new, previously used, or cleaning cartridge may be installed.

Duration	Description
30 seconds	Empties a section of the fluid path from the sippers to the syringes. The remaining fluid in the channel is pushed to waste in the next Recipe Action.
2 minutes	Empties the fluid path and continues to pump air through to dislodge stray droplets and dry the channels

Table 6: *Purge Durations and Corresponding Descriptions*

Formulate: Draws in formulation reagents and pushes the fluid through the fluid path and through the cartridge, using the parameters entered into the selected Recipe.

6 Starting the Blaze Instrument

Start by ensuring the Blaze is plugged into power and connected to the accompanying laptop. The power panel is on the lower left side of the Blaze instrument, and houses the main power supply port, USB port, and power switch.

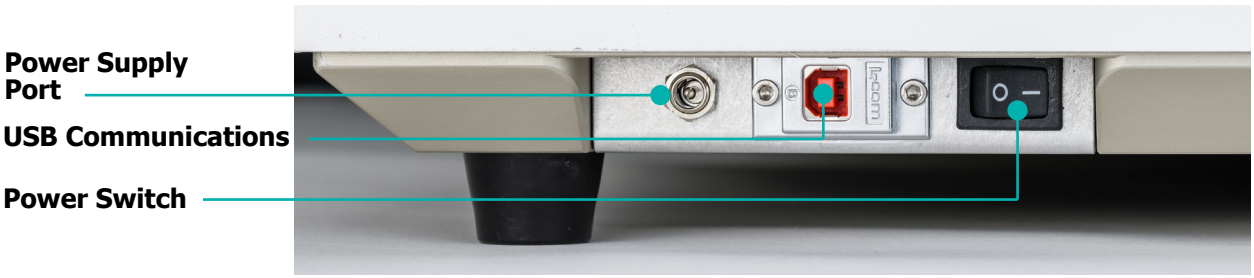


Figure 6-1: *Blaze Power Panel*

Once the power and USB connections have been verified, to turn on the Blaze:

1. Close the lid.
2. Ensure the power cable is plugged into the power supply port and turn on the power switch.
3. Power on the laptop and start the Blaze software by clicking the Blaze Icon.

4. A pop up will appear on the screen with options to **Initialize** or **Recover**. Click **Initialize** if there are no fluids to collect from the fluid path. Alternatively, if there is fluid in the fluid path to collect in the input vessels, click **Recover**. If starting with **Recover**, make sure there are bottles under the input sippers to catch the recovered fluids.

KEY CONSIDERATIONS

Choosing **Initialize** will move all syringes and sippers to the starting position. If there is fluid in the fluid path, choosing this option will result in the fluid being emptied towards the waste container. If no cartridge is installed, fluid may collect above the fluid block.

WARNING! Electrical Hazard: Use only parts supplied by Precision NanoSystems. Do not replace the power cord with one from a third-party vendor. Ensure the instrument is always plugged into a grounded receptacle.

WARNING! Injury Hazard: Do not attempt to override the lid interlock to avoid being injured by moving parts.

WARNING! Injury Hazard: When opening and closing the lid, be careful to avoid hitting a nearby person with it.

7 Blaze Software

The Blaze is controlled by software installed on the accompanying laptop. The Blaze software has two main areas: the top bar and the tabbed page area.

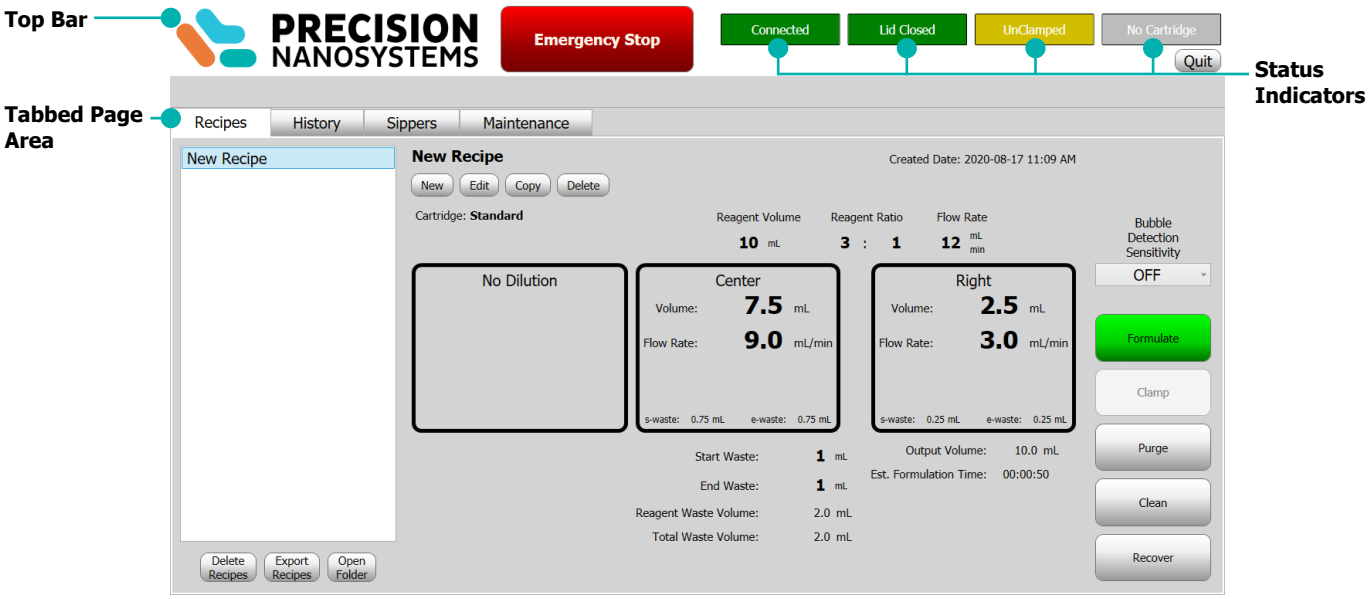


Figure 7-1: *Blaze Software Window*

The top bar has status indicators which give information such as:

- Instrument is "Connected" or "Powered Off"
- Lid is "Open" or "Closed"
- Cartridge is "Clamped" or "UnClamped"
- Cartridge type is "Dilution" or "Non-Dilution"

KEY CONSIDERATIONS

The **Emergency Stop** button will always be at the top of the page. The Emergency Stop will ensure that all operations are stopped, however, the instrument will remain powered-up.

The tabbed page area has four tabs, *Recipes, Sippers, History* and *Maintenance*. The following table gives the purpose of each tab.

Tab Name	Purpose
Recipes	The main page, which opens with the software. Used to create, view, and edit formulation recipes. The bubble sensitivity drop down menu and the buttons for the Recipe Actions described in Section 5 Workflow are on the right hand side of the *Recipes* tab.
History	Shows summary information for previous runs stored in the run history log.
Sippers	Used to raise and lower individual sipper straws when aligning input bottles under the straws.
Maintenance	Provides access to several features that are useful in performing maintenance activities. See Section 13.2 Maintenance Tab for more details.

8 Preparing the Instrument

Prior to formulating, the Blaze needs to be configured to handle different fluid volumes and includes several accessories to handle multiple input and output vessels. These vessels need to be aligned beneath the sippers and outlets. The proper cartridge also needs to be inserted to complete the fluid path.

KEY CONSIDERATION

The Center and Right inputs can be used to input either formulation component. However, the input component vessels must be placed so they match the parameters in the recipe on the Blaze software.

8.1 Installing a Cartridge

The procedure for installing cartridges is the same for all types of Blaze cartridges:

1. Open the lid
2. Use a lint free lab tissue to wipe any fluid or debris around the fluid block exit holes
3. Insert the selected cartridge
4. Push the cartridge in against the white spring-loaded tab
5. Lower the cartridge down into the holder

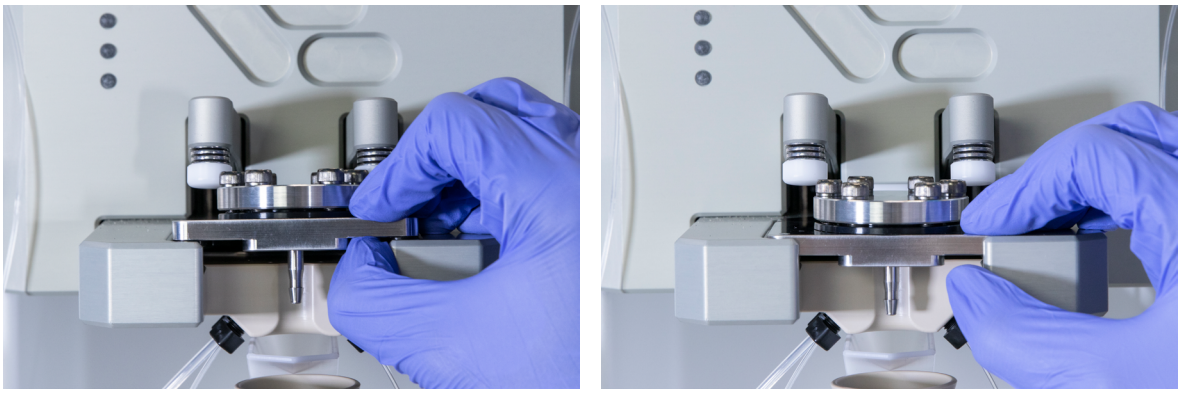


Figure 8-1: *Installing a Blaze Cartridge*

8.2 Input Vessels

Conical centrifuge bottles are best for ensuring the most reagent material can be accessed by the sippers. Bottle supports are supplied to hold 50, 250, and 500 mL conical centrifuge tubes and bottles. The supports have removable top plates for use with different sized bottles.

Figure 8-2: *Bottle Supports*

For larger input volumes, flat-bottomed media bottles can be used. Be aware that more fluid will be left at the bottom of a media bottle than a conical bottle.

The following image shows the typical arrangement of the supplied fluid containers.

Figure 8-3: *Typical Fluid Container Arrangement*

8.2.1 Centering Input Vessels

To ensure the input vessels are centered under the sippers before beginning a formulation, the controls on the *Sippers* tab can be used to lower the sippers into the fluid. Raise the sippers again after performing this test. Ensure the lid is closed throughout.

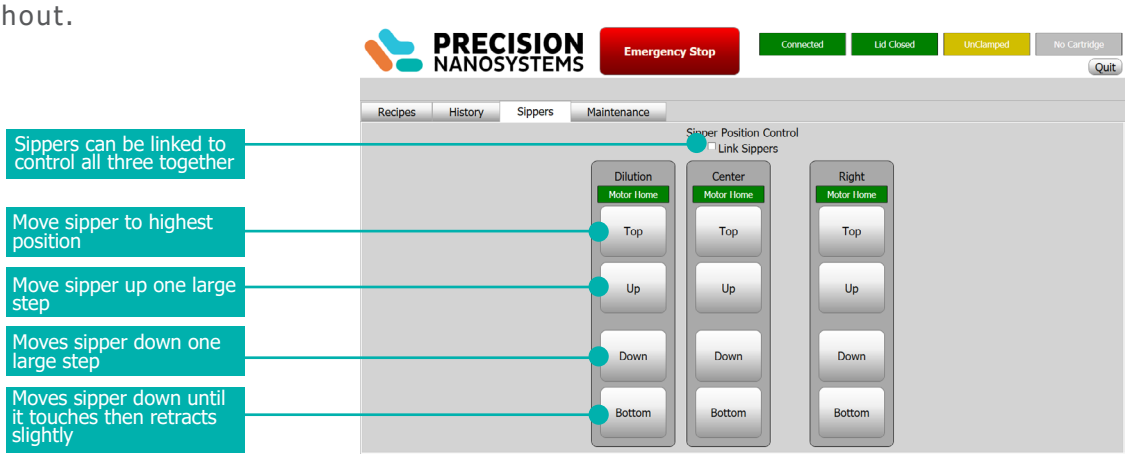


Figure 8-4: *Sippers Tab Controls*

8.3 Output Vessels

The output vessels include the sample collection and waste collection vessels. The sample bottle is positioned under the cartridge outlet. Bottle holders are supplied to support 50, 250, and 500 mL conical centrifuge bottles. For larger volumes, a funnel and media bottle can be used instead of centrifuge bottles. If necessary, use a stand to reduce the distance between the funnel and media bottle.

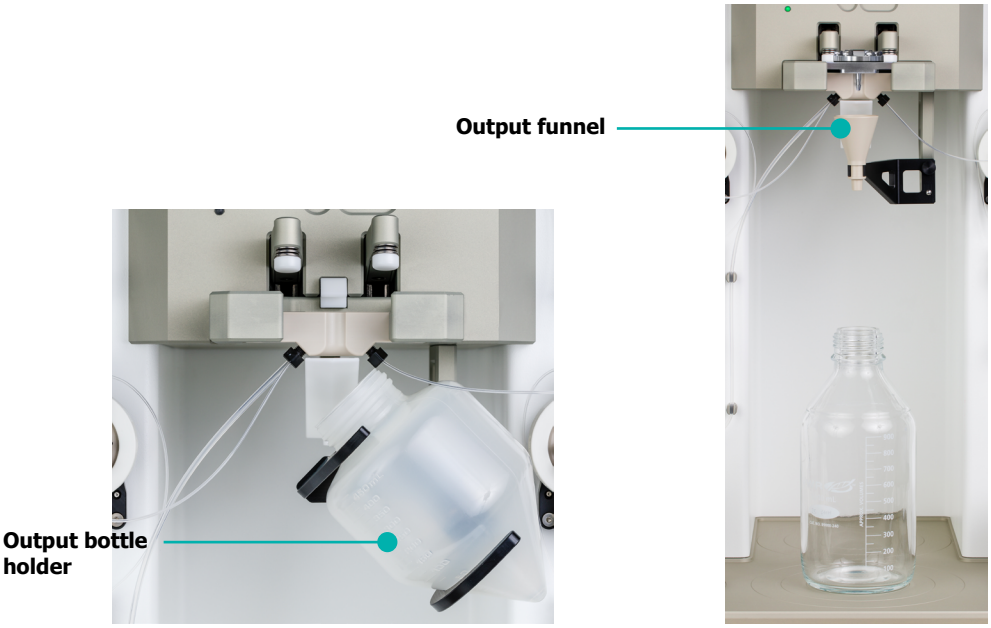


Figure 8-5: *Output Vessel Arrangement*

To switch out or remove a bottle holder, unscrew and remove the thumbscrew attaching the holder, and slide the holder off the pin. Reverse the steps with a different holder.

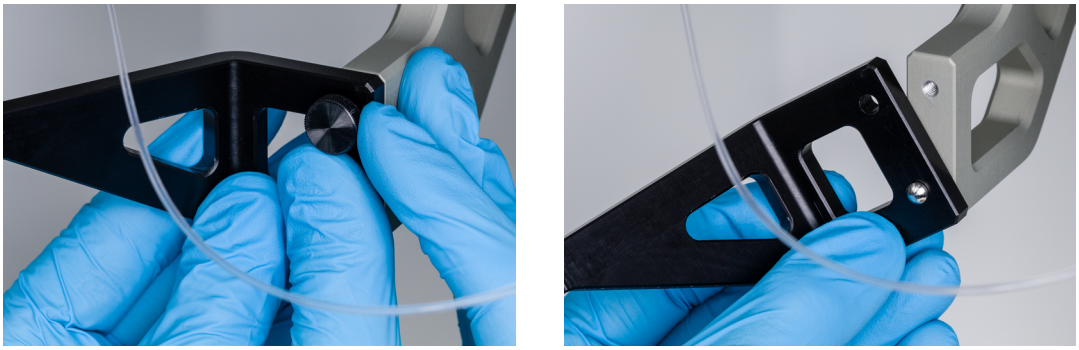


Figure 8-6: *Swapping Output Bottle Holders*

The supplied graduated pitcher can be used as the waste container.

9 Preparing the Fluid Path for a Formulation

WARNING! Chemical Hazard: Before handling a previously unused chemical, read its SDS and make sure to take the necessary precautions.

Either a sanitization or a wash protocol should be performed before or after every formulation. The frequency of either protocol may be adjusted, from a per formulation basis to a daily, weekly, or monthly schedule, dependent on the formulation and the criticality of the experiment. More information can be found in Section 9.2 Recommended Sanitization Protocol and Section 9.3 Recommended Wash Protocol.

In some cases, a full sanitization is unnecessary. For instance, if multiple iterations of the same formulation or formulation type are being performed in a single day, a much simpler wash protocol is sufficient. This decision ultimately depends on the level of risk mitigation necessary for the studies being performed.

The Blaze fluid path should be sanitized and stored dry when left for an extended period, in order to minimize any residue or contamination that can occur in the fluid path. When preparing for a formulation and the previous formulation activity is unknown, it is recommended to sanitize the fluid path prior to formulating.

KEY CONSIDERATIONS

After a sanitization or wash protocol is performed, an optional **Clean** of the fluid path with the respective formulation solvents may be necessary. This step ensures that any small pockets of water retained in the fluid path are replaced with formulation solvents to avoid potential precipitation during formulation. This flush should also be followed by a **Recover** prior to formulation.

9.1 Initializing the Fluid Path on Blaze

Generally, the Blaze has three Recipe Actions to enable the washing and sanitization protocols: **Clean**, **Purge**, and **Recover**. Refer to Section 5 Workflow for more details on these Recipe Actions.

To perform a Recipe Action on Blaze:

1. Wipe the outside of the sippers with ethanol.
2. Insert and **Clamp** a cartridge if needed. (See Section 8.1 Installing a Cartridge) Both a **Purge** and **Clean** require a cartridge to be installed, but a **Recover** does not. A cleaning cartridge is supplied with each Blaze instrument and can be used here. Alternatively, a regular Classic or NxGen cartridge can be installed. The dilution channel will only be cleaned or purged if a corresponding dilution cartridge is inserted.
3. Place input containers of an appropriate volume under the sippers as needed. The volume requirement is dependent on the duration of the Recipe Action selected – refer to Section 5 Workflow.

4. Place a waste collection vessel under the waste output if running a **Purge** or **Clean**. If running a **Recover**, place collection vessels under the sippers.
5. Close the lid.
6. Ensure bottles are centered below each sipper. See Section 8.2.1 Centering Input Vessels.
7. Click the relevant Recipe Action button and check all the boxes on the corresponding Recipe Action tab, ensuring each item has been performed (shown in Figure 9-1).
8. Select a duration for the Recipe Action.

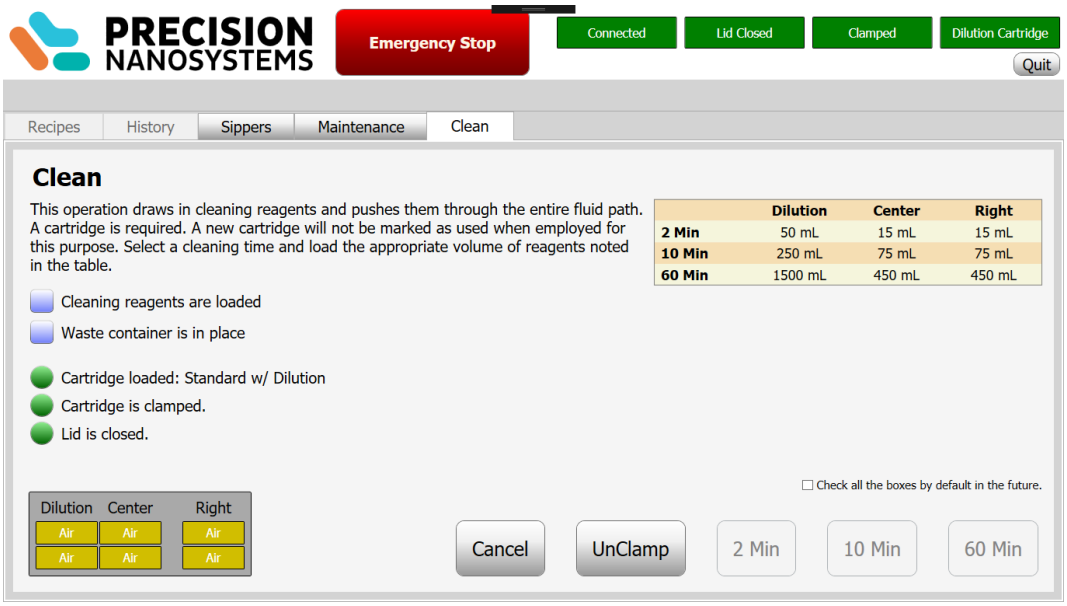


Figure 9-1: *Cleaning Tab*

9. Monitor the Recipe Action by ensuring the bubble detector status on the bottom left of the screen updates to "Air" for a **Recover** or **Purge**, and to "No Air" for a **Clean**.
10. When complete, the lid may be opened to optionally remove the cartridge, input, and waste containers.

9.2 Recommended Sanitization Protocol

A sanitization protocol should be performed when risk mitigation is high or if contamination is likely. For example, this step may be necessary if a critical animal study is being performed or if different formulations are running sequentially.

Washing protocols used in high-performance liquid chromatography typically use 10 times the fluid path volume. We follow the same standard for sanitizing and washing the Blaze. The volume of each input path is about 5 mL for the Center and Right channels and 25 mL for the dilution channel, so 10 times that is about 50 mL and 250 mL respectively (See Table 4). To make sure the lines do not run dry, we recommend 60 ml and 260 mL respectively when using conical centrifuge bottles.

The following sanitization protocol is recommended but may be adapted as needed:

Step	Recipe Action	Duration	Fluid
1	Purge	2 mins	n/a
2	Clean	10 mins	60 mL Nuclease-free water
3	Purge	2 mins	n/a
4	Clean	10 mins	60 mL 0.5N NaOH
5	Purge	2 mins	n/a
6	Clean	10 mins	60 mL Nuclease-free water
7	Purge	2 mins	n/a
8	Clean	10 mins	60 mL 25 nmol/L acidic buffer with pH 3-6
9	Purge	2 mins	n/a
10	Clean	10 mins	60 mL Nuclease-free water
11	Recover	2 mins	n/a

Table 7: *Recommended Sanitization Protocol*

KEY CONSIDERATIONS

A dilution input is required if a dilution cartridge is used in the protocol. Each step in the sanitization protocol requires 260 mL of solution in the dilution input when using a dilution cartridge. See Table 4.

A **Recover** is the best way to remove fluid from the fluid path and should always precede a formulation.

9.3 Recommended Wash Protocol

A wash is a much faster and simpler method to reduce the risk of contamination between formulations. It may be used between formulations of a similar type or payload, or as necessary. A wash relies on repeated dilution as the primary means of contamination reduction.

The following wash protocol is recommended but may be adapted as needed:

Step	Recipe Action	Duration	Dilution Fluid	Center Fluid	Right Fluid
1	Purge	2 mins	n/a	n/a	n/a
2	Clean	10 mins	260 mL Dilution buffer	60 mL Aqueous buffer	60 mL Organic solvent
3	Clean	10 mins	260 mL Nuclease-free water	60 mL Nuclease-free water	60 mL Ethanol
4	Clean	10 mins	260 mL Nuclease-free water	60 mL Nuclease-free water	60 mL Nuclease-free water
5	Recover	2 mins	n/a	n/a	n/a

Table 8: *Recommended Wash Protocol*

KEY CONSIDERATION

Table 8 is written with the assumption that the Center fluid channel contains the aqueous component and the Right fluid channel contains the organic component. The wash protocol may need to be adapted for other formulations such as formulations with a polymer component or with aqueous/aqueous mixing. The dilution input is needed only when a dilution cartridge is used in the protocol.

10 Formulating on Blaze

Once the reagent containers, cartridge, and waste container have been inserted and the fluid path has been prepped, the Blaze system is ready to start a formulation. The system formulates using Recipes—a named and saved set of formulation parameters. This feature allows for Recipe re-use, logging, and storage.

10.1 Creating a Recipe

Recipes are simple to create. They require all the familiar formulation parameters used on the rest of the NanoAssemblr Platform. See Section 4.5 Glossary of System Parameters.

To create a recipe, click **New** on the *Recipes* tab.

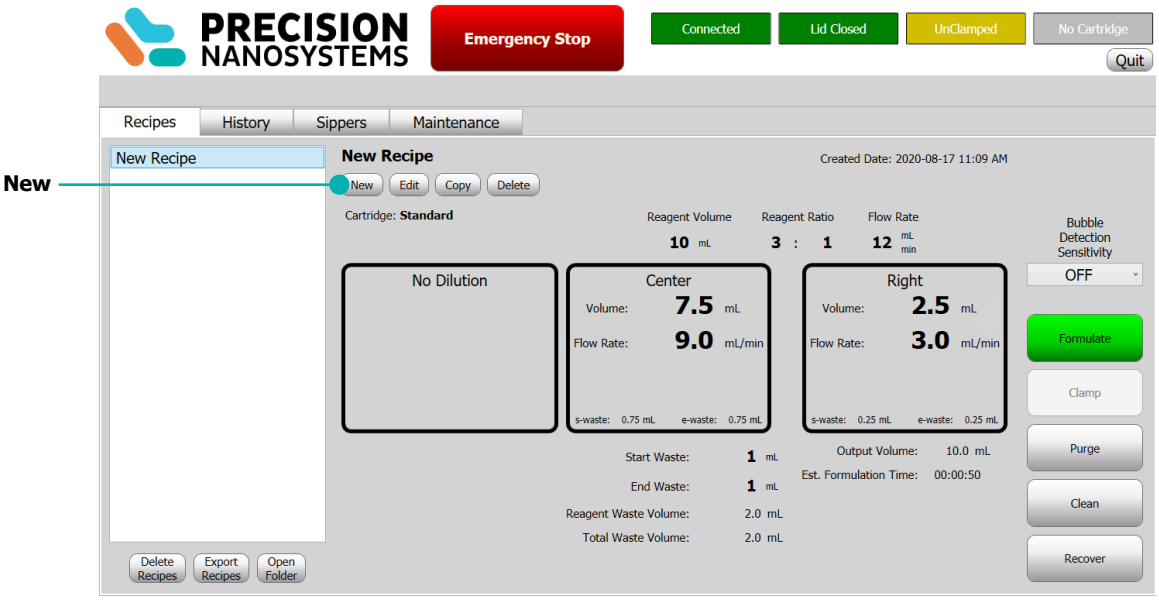


Figure 10-1: *Blaze Recipe Home Screen*

A pop-up will appear allowing the:

- Entry of the Recipe Name
- Selection of the Cartridge Type

Cartridge Type	For use with
Standard	Classic cartridge
Standard w/ dilution	Classic cartridge with dilution
High Flow	NxGen cartridge
High Flow w/ Dilution	NxGen cartridge with dilution

Table 9: *Cartridge Types*

- Input of the Reagent Volume, Reagent Ratio and Flow Rate
- Input of the Dilution Ratio and selection for using the dilution sipper, if a dilution cartridge type has been selected
- Entry of the Start Waste and End Waste. See Section 10.1.1 Determining Waste to determine a suitable volume.

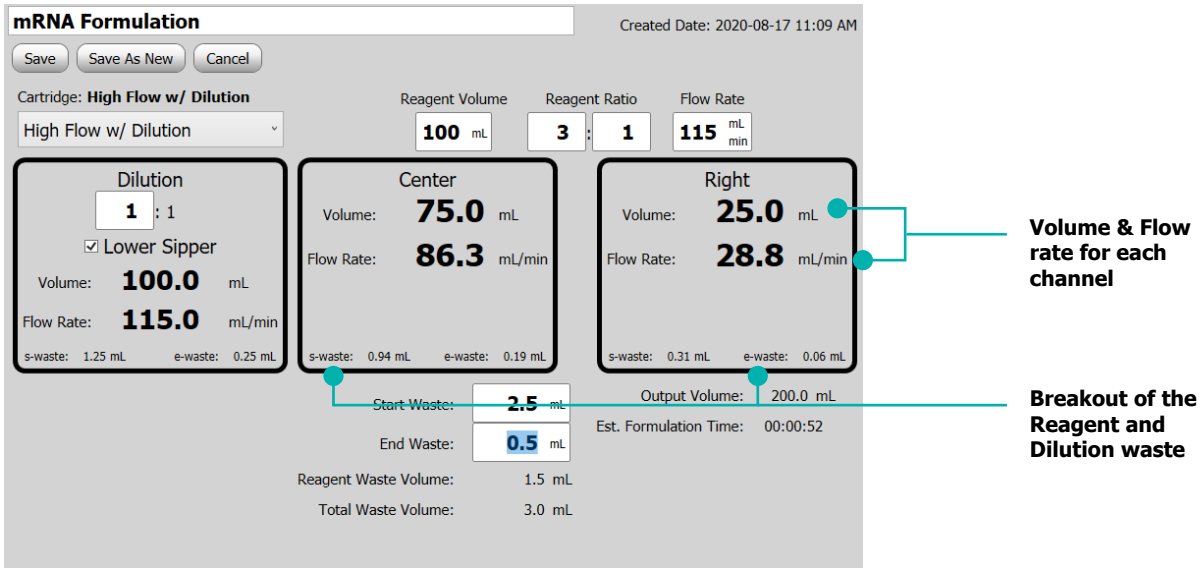


Figure 10-2: *Recipe Editing Screen*

The screen will then display the volume and flow rate for each channel, along with the breakout of the Reagent and Dilution Waste. Any of the above parameters can be edited for a formulation.

Click **Save**; the recipe can now be accessed from the left panel of the *Recipes* tab as seen in Figure 10-1: Blaze Recipe Home Screen. Recipes can be edited, copied or deleted from the *Recipes* tab also.

KEY CONSIDERATION

An error can occur when inputting the formulation parameters if the parameters fall outside the recommended parameters listed in the Section 2 Specifications. To avoid these errors, stay within the allowable parameters.

10.1.1 Determining Waste

Waste collection is intended to collect the small portions at the beginning or end of a formulation where off-target conditions can occur. These non-steady-state flow conditions may be the result of system acceleration or improper reagent loading. Off-target flow rates and ratios may yield nanoparticles with bimodal populations or unexpected characteristics.

Unlike other systems of a similar scale, the Blaze has been optimized to reduce waste volume and is able to collect a defined amount of Start Waste and End Waste, respectively.

10.1.1.1 Start Waste

Start Waste is collected in the waste container at the very beginning of a formulation. A user-specified volume input on the *Recipes* tab is collected from the combined Center and Right channels, and Dilution channel if it is being used.

Blaze has been designed to reduce Start Waste by minimizing fluid path compliance and accelerating to the target Total Flow Rate and Flow Rate Ratio very quickly. Start Waste volumes on Blaze do not have any volume dependencies. Given the same Total Flow Rate, Flow Rate Ratio, and Dilution Ratio (if necessary), the Start Waste will remain the same for any given output volume.

When inputting the Start Waste, consider the breakout of the volumes amongst the Dilution, Center and Right Channels. Ensure the **Start Waste volume is at least 0.3 mL** for the channel with the lowest flow rate.

10.1.1.2 End Waste

At the end of a formulation, the syringes come to a complete stop very quickly, greatly reducing End Waste. **An End Waste value of 0.5 mL or less is recommended.**

10.2 Bubble Detection

Blaze is also equipped with bubble detectors to facilitate fluid path priming and bubble detection. The bubble detectors can detect bubbles in the fluid path during a formulation, and end the formulation based on the user selected sensitivity. The applied setting remains for all Recipes until it is updated by the user.

Sensitivity Setting	Volume of Air Detected to end the Formulation
High, Medium	< 300 µL
Low	> 300 µL
Off	NA

Table 10: *Bubble Detection Sensitivity*

The Low sensitivity setting is recommended.

If the bubble sensitively is set to "Off", a warning will appear, but the formulation will proceed as normal. This can lead to off target mixing if an input reagent runs dry.

10.3 Running a Formulation

Before continuing with a formulation, ensure the fluid path has been adequately prepared and the channels have been flushed with the appropriate solvents by running a **Clean**.

1. Open the lid and install the cartridge that will be used for formulating if it is not already installed. See Section 8.1 Installing a Cartridge.
2. Insert the formulation input vessels. Ensure bottles are centered below each sipper. See Section 8.2.1 Centering Input Vessels. Make sure the Center and Right input bottles match with the recipe being used.

> **KEY CONSIDERATIONS**
>
> The hold-up volume for each channel is approximately 5 mL. The hold-up volume is the volume of the fluid path for an individual input channel. To ensure there is no off-target mixing, an additional 5 mL of each input solution should be included in each input vessel. This will prevent channels running dry before the formulation is complete.

3. Place the sample and waste collection vessels appropriately.
4. Close the lid
5. **Clamp** the cartridge using the Blaze software.
6. Select the Recipe from the list in the *Recipes* tab and select **Formulate**.
7. A new *Formulation* tab will open. In the *Formulation* tab check all the boxes, ensuring each item has been performed.
8. Confirm the Recipe parameters on the right panel, ensuring extra volume has been added to account for the fluid path hold-up volume. These additional volumes are factored in on the displayed "Input Volumes (mL)" row.

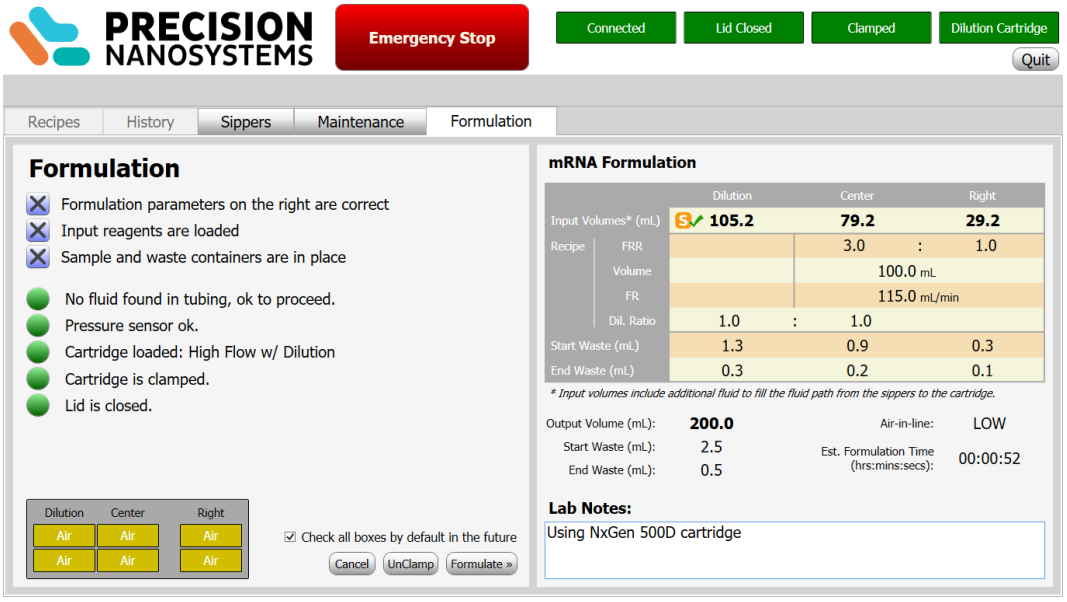


Figure 10-3: *Formulation Screen with Recipe Parameters Displayed on the Right Panel*

9. Enter any additional details about the formulation in the Lab Notes text box, which will get saved with the formulation details in the History file. Lab Notes can also be used to record the cartridge type being used.
10. Select **Formulate**.

10.4 Monitoring the Formulation

After starting a formulation, the sippers lower into the input bottles. The Blaze system will then perform four steps as indicated by the status bar:

1. **Preload input lines**: Fluid is drawn into the fluid path to preload the input channels, until fluid is detected
2. **Finding fluid**: Continues to draw fluid into fluid path until fluid is detected
3. **Priming the cartridge**: Flushing reagent fluid through the fluid path to the cartridge.
4. **Formulating**: Fluid will begin to exit the cartridge to be collected.

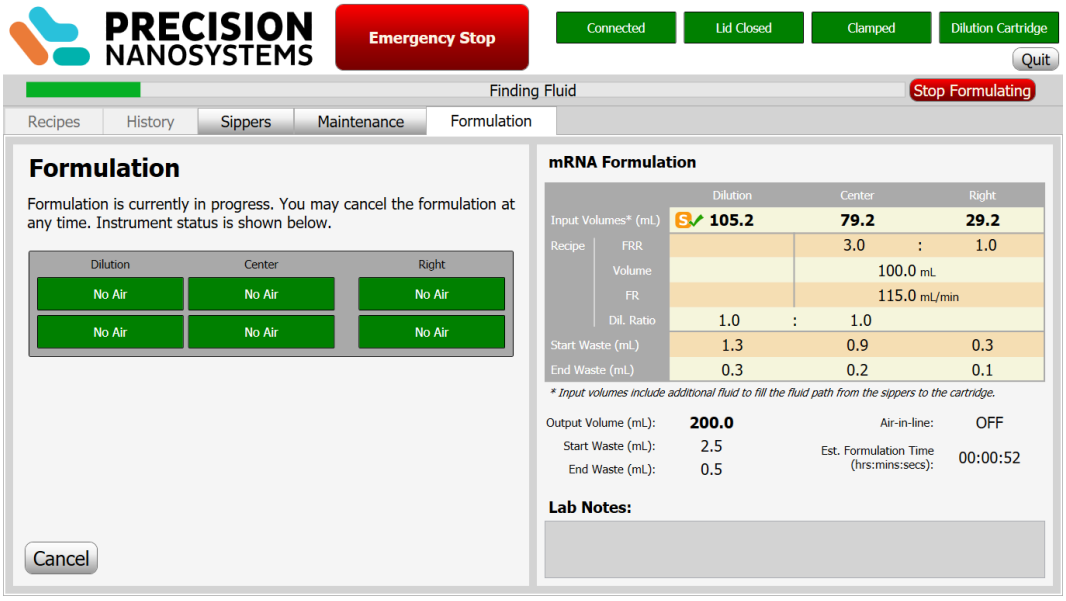


Figure 10-4: *Formulation Screen During an Active Formulation*

KEY CONSIDERATIONS

Once the progress bar message indicates "Formulating," the cartridge cannot be used in another formulation.

Be sure to observe the instrument status to ensure it changes from "Air" to "No Air" during the formulation.

Once the status bar updates to "Formulating," the Start Waste will exit the cartridge and be collected by the waste collector. After the starting waste volume has been collected, the waste collector will then retract, and the sample will start flowing into the collection vessel underneath the cartridge output.

When the reagent volume has been formulated, the waste collector will move out to collect the End Waste, and the screen will update to indicate that the formulation has been completed.

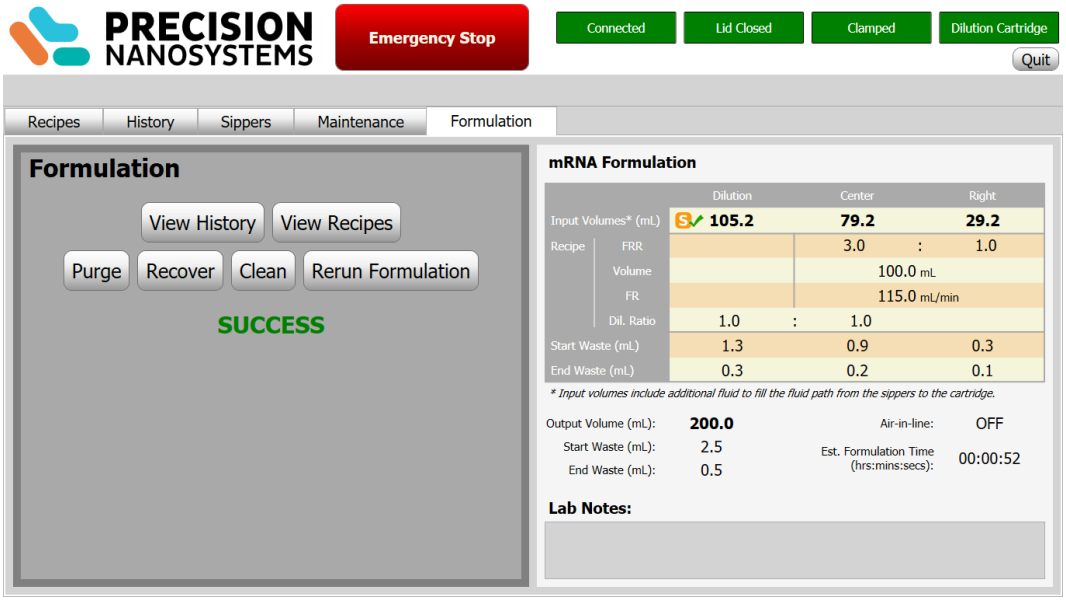


Figure 10-5: *Formulation Screen after a Successful* ***Formulate***

10.5 Cancelling a Formulation

A formulation or any other Recipe Action can be cancelled by clicking the **Stop** button beside the status bar on the top right, or the **Cancel** button on the bottom left of the screen. This will cancel the Recipe Action and allow the user to perform a **Purge** or **Recover** afterwards.

If the formulation is cancelled by clicking the **Emergency Stop** button, or by opening the lid, a pop-up screen will appear giving the options to **Initialize** or run a **Recover**.

11 Post-Formulation

If there are any spills or drips, wipe them up immediately.

After a formulation, fluid will remain in the fluid path. A **Recover** or **Purge** will remove the material. It is best to run a wash or sanitization protocol to ensure there is no buildup of material or degradation in the fluid path. Refer to Section 9 Preparing the Fluid Path for a Formulation for more details.

A detailed log of each formulation performed is maintained. This information, along with Recipe details, may be exported as needed.

11.1 History

To review the formulation parameters and performance after the formulation, click on the *History* tab. The following details are recorded:

- Date and Time
- Recipe Name
- Mixing Parameters
- Formulation Outcome (Success/Cancelled/Error)
- Lab Notes
- Pressure Graph

The cartridge type is not recorded as part of this log. To save this information in the history file, add a comment with the cartridge type to the Lab Notes on the *Formulation* tab when formulating.

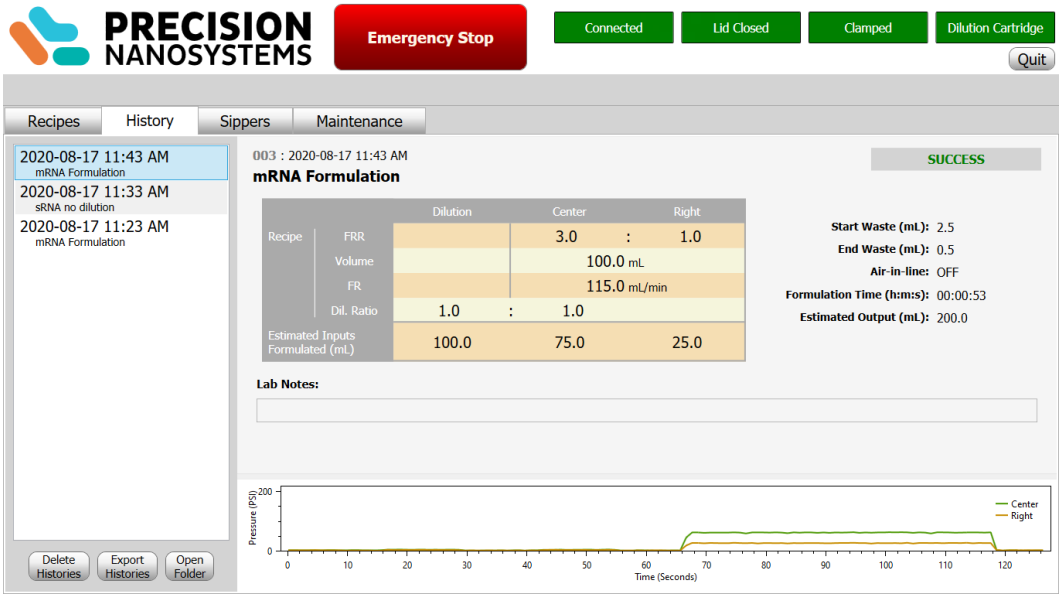


Figure 11-1: *History Screen*

The history log displays pressure information recorded during the formulation. High pressures can point towards increased restrictions in the fluid path due to material build-up or some other blockages. This feature can be useful in determining the suitability of a formulation for larger scale experiments and can point towards further opportunities for formulation optimization.

11.2 Exporting Information

Information can be exported from both the *Recipes* and *History* tabs in the Blaze software. This information can be stored external to the Blaze system for further analysis or storage.

11.2.1 Exporting Recipes

Recipes can be exported as .txt files. To export Recipes:

1. Open the *Recipes* tab
2. Click **Export Recipes** underneath the list of recipes on the left panel
3. Select the Recipes to be exported
4. Click **Export Text**
5. A file explorer screen will open, with the default file path being: C:\Users\NanoAssemblr Blaze\Documents\Precision Nanosystems\Recipes
6. Choose another file path if desired and enter a file name.
7. Click **Save**

The exported Recipes can be accessed by clicking **Open Folder** underneath the list of recipes on the left panel. The text files will contain the following information:

- Recipe Name
- Creation Date
- Reagent Volume
- Total Flow Rate
- Flow Ratio (C)
- Flow Ratio (R)
- Amount of Start Waste
- Amount of End Waste
- Dilution Sipper usage
- Flow Ratio (D)
- Diluted Volume

11.2.2 Exporting Histories

Histories can be exported as either .txt or .csv files. To export Histories:

1. Open the *History* tab
2. Click **Export History** underneath the list of recipes on the left panel
3. Select the Recipes to be exported
4. Choose to export “To Text” or “To CSV”, depending on the preferred file format
5. A file explorer screen will open, with the default file path being: C:\Users\NanoAssemblr Blaze\Documents\Precision Nanosystems\History

6. Choose another file path if desired and input a file name
7. Click **Save**

The exported History can be accessed by clicking **Open Folder** underneath the list of History records on the left panel. The file will contain the following information for each formulation:

- Formulation Date & Time
- Formulation Name
- Result of Formulation
- Lab Notes
- Recipe details
- Bubble Detection sensitivity setting
- Formulation ID
- Actual Run Time
- Estimated Fluid Used across D – C – R

11.3 Shutting Down

The Blaze can be shut down or left on at the end of the day. To shut down the Blaze, follow these steps:

1. Run a wash or sanitization protocol if it was not done following the last Formulation
2. In the software, select **Quit**, and then on the prompt select **Really Quit?**
3. Shut down the computer from the Windows operating system.
4. Switch off the Blaze at the power Panel.

12 Troubleshooting

The Blaze has been designed for ease of use and provides simple instructions for errors where possible. As a further reference, errors that may occur and their remedies are described below:

Error Message	Solution
Not Connected	1. Check that the instrument is powered up. 2. Check that the instrument is connected to the laptop. 3. Click **Connect Blaze Instrument** on top right corner of screen.
Interrupt	1. Ensure the lid is closed. 2. Select **Initialize** or **Recover** — choose **Initialize** when there is no fluid in the fluid path and choose **Recover** when there is fluid to collect.
Fluid Detected	1. Check the status of the fluid detection on the formulation screen to determine which bubble detector is reporting "No Air". 2. Inspect the relevant channel(s) for fluid. 3. If the channels are full, do not continue. Click **No** on the pop-up screen, then run a **Purge** or **Recover** to empty the channels. If the channels have been emptied already, stray droplets may remain in the channel, triggering the bubble detector. In this case, operation may continue.
Dilution channel has fluid, non-dilution cartridge loaded	1. Check the status of the fluid detection on the *Formulation* tab to determine which bubble detector is reporting "No Air". 2. Inspect the relevant channel(s) for fluid. 3. If fluid remains in the dilution fluid path, it is worth emptying it with a **Recover** or **Purge**. If the channel has been emptied already, stray droplets may remain in the channel, and it is not necessary to run a **Recover** or **Purge**. Click **Yes** on the pop-up to continue with a non-dilution purge. Note that a Fluid Detected error message will appear when the next dilution formulation is run.

Table continues on next page...

Air detected in the Fluid Path	When the bubble detectors sense air in the channel during a formulation, the system stops all intakes and finishes dispensing the last full syringe strokes. The formulation finished screen is shown with an error message indicating that it was stopped early, and which channel ran out of input fluid. 1. Increase volume of fluid prepared for that channel if the formulation is ever re-run.
Overpressure	1. Select **Unclamp** on the pop-up screen. 2. Open the lid and uninstall the cartridge. 3. Close the lid and select **Initialize** or **Recover** — choose **Initialize** when there is no fluid in the fluid path and choose **Recover** when there is fluid to collect. 4. In the *Maintenance* tab, check the Center and Right pressure readouts. 5. Both should read between -10 and 10 psi — if not, contact your local Field Application Scientist. 6. If the readouts are within this range, it is likely the cartridge caused high resistance, resulting in an overpressure event. 7. If the overpressure event occurred during a formulation, check the *History* tab to determine if the pressure increased throughout the Formulation, exceeding 150 psi. Contact your local Field Application Scientist for advice on Microfluidic Cartridge selection.

Table 11: *Error Messages and Solutions*

For any other issues or errors not covered above, contact your regional Field Application Scientist or email us at support@precision-nano.com.

13 Safety, Cleaning, and Maintenance

13.1 Safety

13.1.1 Symbols

Symbol	Description
	Warning, risk of danger. Documentation should be consulted. Proceed with caution
	Warning, crush hazard. Keep hands clear from moving parts to avoid pinch point.
	Warning, flammable material. Materials used may be highly flammable when subject to heat. See section 13.1.2 for more information.
	Warning, biological hazard. Indicated hazards are dependent on the materials selected by the User. Refer to SDS and take appropriate precautions for the materials used.
	Warning, electrical shock/electrocution. Risk of serious injury or death if instrument is energized while panels and access doors are opened. Always disconnect power sources and follow proper safety procedures to prevent electric shock.
	Warning, overhead obstacles. Risk of injury. Exercise caution when working in vicinity of the instrument with the front enclosure in open position.
	Warning, lifting hazard. Heavy object, risk of injury while lifting. Seek the assistance of a second person and use proper techniques while lifting to avoid injury.

Table 12: *Safety Symbols*

13.1.2 Important Safety Information

WARNING! Injury Hazard.

Except for procedures given in this guide do not try to service or replace parts on the instrument. For user safety and to protect the instrument warranty, contact Technical Support with any service requests.

To maintain Blaze, regular cleanings and a preventative maintenance service are recommended. Blaze has enabled features to facilitate cleaning and maintenance activities.

WARNING! Flammable Material.

Organic solvents are highly flammable. When using the system with flammable liquids, users should conduct a risk assessment and always follow basic safety precautions to reduce the risk of electric shock, fire, and injury to a person, including the following:

- Ensure that suitable precautions are in place to safely handle hazardous liquids and that any potential spills can be contained and cleaned up in a safe manner.
    - When operating the system with large quantities of flammable liquids, it is recommended that the system remain under supervision during the duration of the run, in order to respond to any potential leaks by stopping the system, isolating the leak, and/or providing safe containment.
    - When operating the Blaze+ with external vessels, vessels should have a shut-off close to the source to ensure that leaks can be isolated quickly and that the resulting spill volume is minimized.
    - Personal protective equipment including safety glasses and gloves should be worn during all work with the system.
- Ensure that work areas are always well-ventilated.
- Remove any external ignition sources or open flames in the vicinity of the unit when operating the unit with flammable liquids.
- Users should take suitable precautions to avoid the potential ignition source of static discharge from the operator. The system may be wiped down with a damp cloth to avoid static buildup that may occur in dry ambient conditions.
- Electrostatic charge build up may occur as fluids pass through the system, and if the final formulation composition is such that an ignitable vapor cloud can form, users should take precautions when handling the fluid to prevent an ignition from static discharge.
- Users should ensure that collection vessels are appropriately sized for a given formulation program.
- Depending on fluid properties and chosen recipe parameters the collected waste and formulation liquids may still be flammable. When formulation is complete, handle the collection vessels appropriately.
- When operating the Blaze+™ with the closed system output assembly, ensure that all inlet valves on the collection vessel are in the open position.
- The system should only be power cycled in the absence of any flammable liquids or vapors in the immediate area.

Do not use the system in potentially explosive atmospheres without appropriate precautions and protective equipment. As appropriate, consult local authorities regarding local rules and regulations before installing or operating.

13.2 Cleaning the Blaze Instrument

Should any material be spilled or dripped inside the instrument, immediate clean-up is recommended. Cleaning can also be performed as a preventative measure.

The instrument body, lid, and all components may be wiped down with warm water and a mild non-abrasive detergent; ethanol or isopropyl alcohol may also be used. The lid should be wiped with a soft cloth to avoid scratches that could impact visibility.

13.3 Maintenance Tab

The *Maintenance* tab provides access to several features that may aid in performing cleaning or maintenance activities.

Under the *Maintenance* tab, users have access to

- Pump controls
- Status indicators
- Waste Collector and Clamp controls
- Pressure readouts
- Software and firmware version
- Access to the Log Directory

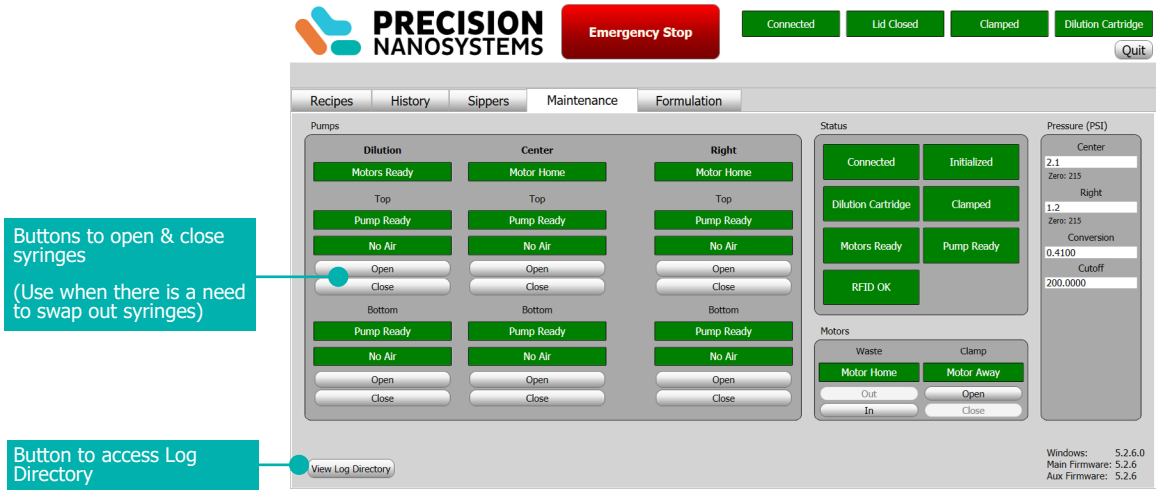


Figure 13-1: *Maintenance Screen*

13.4 Replacing Syringes

Should the syringes require replacement due to wear, follow the steps outlined here. Only use syringes approved to work on the Blaze; replacements can be ordered if necessary. To replace a syringe:

1. On the *Maintenance* tab, click **Open** for the relevant syringe
2. Unscrew (counterclockwise) the syringe thumbscrew – use pliers if needed
3. Push the syringe plunger up to the closed position
4. Unscrew (counterclockwise) the syringe from the valve at the valve-syringe port

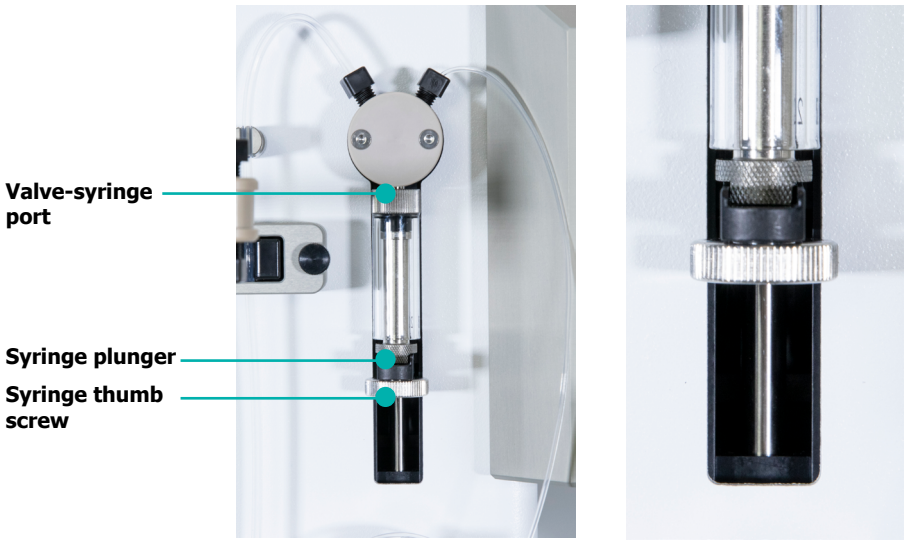


Figure 13-2: *Syringe Components*

5. Screw in a new syringe into the valve-syringe port
6. Pull the plunger down to the syringe driver — ensure the plunger base is positioned correctly
7. Secure the syringe plunger in place by tightening the syringe thumbscrew
8. Ensure there is no gap between the bottom face of the syringe, the syringe driver and the thumbscrew
9. Click **Close** for the relevant syringe

13.5 Replacing Sippers

If a sipper becomes damaged or bent, it will need to be replaced. A spare set of sippers is included with every Blaze instrument; more may be ordered if necessary. To replace a sipper:

1. Hold the upper fluid connector steady.
2. Turn the lower connector in a counterclockwise direction to loosen.
3. Remove the sipper.
4. Reverse the process to connect the new sipper.
5. Tighten the connector to finger tight.

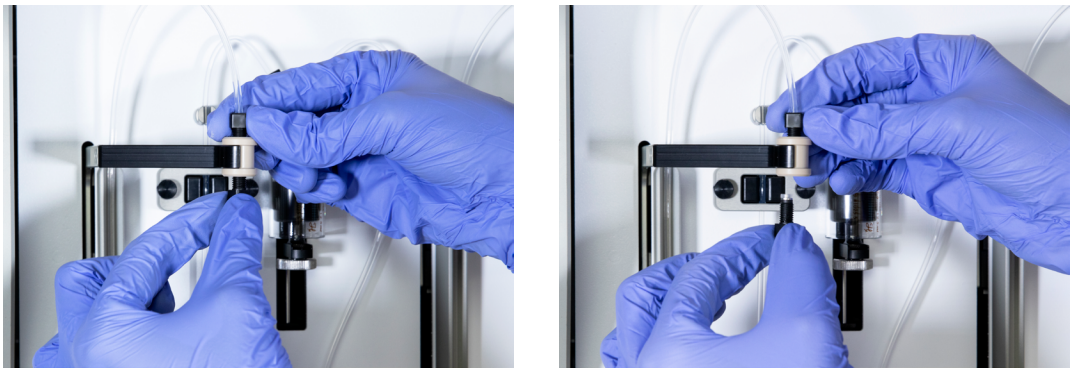


Figure 13-3: *Replacing Sippers*

13.6 Moving the Blaze

If the Blaze system needs to be moved for any reason, it will need to be recalibrated. Contact your Field Application Scientist to arrange for the movement of the Blaze instrument. Correct operation and safety features cannot be guaranteed if they system is moved from its original position without a Precision Nanosystem representative completing a verification and calibration procedure.

WARNING! Lifting Injury Hazard: Do not attempt to move the instrument without a suitable team of people. Contact your Field Application Scientist or email us at support@precision-nano.com for assistance.

13.7 Instrument Disposal

Follow country, state, and local e-waste regulations when disposing of any electronic equipment.

14 Chemical Compatibility

When formulating on the Blaze, there are three fluid-contacting elements: the input and collection vessels, the Blaze Cartridge, and the fluid path. It is important to ensure the chemical compatibility of the reagents with these elements to avoid issues when formulating.

14.1 Input and Collection Vessels

Please consult the manufacturer of the sample collection vessels for chemical compatibility and appropriate level of cleanliness. Consider the compatibility of the input and collection vessels with the mixture of solvents present in the final formulation.

14.2 Cartridges

The Blaze cartridges have been designed to reduce the amount of fluid contacting materials. It is important to consider these materials when running formulations on the Blaze system and to confirm compatibility with the reagents used. The fluid contacting materials used in both cartridge types are outlined here.

Classic: Composed of polypropylene (PP), ethylene propylene diene monomer (EPDM) and a cyclo olefin polymer (COP).

The Classic Cartridge with Dilution has additional tubing that contacts the dilution fluid only, this is made of a plastic blend (Versilon).

NxGen: Composed of stainless steel (SS), ethylene propylene diene monomer (EPDM) and a cyclo olefin polymer (COP).

The microfluidic mixers are composed of COP, which offers good resistance to a wide range of solvents, including acids and bases. A partial list of solvents to which Classic and NxGen Blaze cartridges exhibit good chemical resistance is provided below.

Solvents	Aqueous Solutions
Alcohol, Ethyl	Acid, Acetic 99%
Alcohol, Iso-propyl	Sodium Hydroxide solution
Alcohol, Methyl	Saline
Acetone	Ammonia, 28%
DMF (N,N-dimethylformamide)	Formamide, 50%
DMSO	Glycerine
Methyl Cellosolve	Urea, 50%

Table continues on next page...

Solvents	Aqueous Solutions
Oil, Fluorinated	Tween 20 10% solution
Silicon oil, Dimethylpolysiloxane	Tween 80 10% solution
Silicone oil, Methyl Phenyl	Polyethylene Glycol (PEG4000S, 50%)

Table 13: *List of Solvents and Aqueous Solutions that COP Exhibits Good Chemical Resistance*

This list is not exhaustive due to the multitude of possible conditions including exposure time, temperature, pressure, and other acceptance criteria. It is impractical to test all solvents under all conditions. Solvents not on this list may be tested at the user's discretion under conditions representative of the intended use. Caution should be taken with oils and solvents of low polarity.

14.3 The Fluid Path

The fluid path contains some additional fluid contacting materials to be considered when running formulations on the Blaze system, listed below.

Material
Borosilicate Glass
Stainless steel
Ethylene propylene diene monomer (EPDM)
Fluorinated ethylene propylene (FEP)
Polychlrotrifluoroethylene (PCTFE)
Polyether ether ketone (PEEK)
Perfluoroalkoxy alkane (PFA)
Polytetrafluoroethylene (PTFE)
Ultra-high-molecular-weight polyethylene (UHMW-PE)

Table 14: *Fluid Path Materials*

This is not an exhaustive list, and materials used in the Blaze fluid path are subject to change. It is recommended that you perform material compatibility studies with the solvents and aqueous solutions used in your process before formulating on the Blaze.

14.4 Blaze+ Chemical Compatibility

The Blaze+ system requires additional tubing and connections to bring fluid from external vessels to the fluid path. These additional materials should be considered when performing a chemical compatibility assessment.

Material
Polypropylene (PP)
Platinum-cured silicone
Polycarbonate (PC) – for Closed Output System ONLY

Table 15: *Additional fluid contacting materials for Blaze+*

15 Blaze+

The NanoAssemblr Blaze+ System expands the capabilities of the base Blaze System by enabling nanoparticle assembly of formulation volumes up to 10L, and up to 40L of diluted volume. Using the NanoAssemblr Blaze+ System, users can connect external vessels such as bioprocessing bags to the fluid path. The NanoAssemblr Blaze+ System upgrade modifies the Blaze system with mechanical features that allow for external connections.

KEY CONSIDERATION

The full Blaze User Guide and Blaze+ section should be read before using the Blaze+.

15.1 NanoAssemblr Blaze+ Features

During normal operation, the Blaze+ differs from the Blaze in the following ways:

1. **Fluid Pass Throughs**
   In order to connect external vessels to the Blaze+ fluid path inside the instrument, the tubing will extend outside the Blaze System through a gap at the bottom of the lid. Tube clips are present along the base of the instrument to secure the tubing and protect he tubing from being pinched.

Figure 15-1: *Lid Adjustment and Added Tube Clip.*

The lid must remain closed during normal operation to safe operation. The instrument will not operate unless the lid is closed, and the safety switches are engaged. If the safety switches are not engaged, the software will read "Lid Open."

WARNING! Injury Hazard: Do not attempt to override the lid interlock to avoid being injured by moving parts.

2. **Tubing Kit Required**
   The NanoAssemblr Blaze+ Tubing Kit provides single use tubing assemblies with fittings that connect larger externally located vessels to the Blaze+ system. The tubing kit contains three input tubing assemblies for Dilution, Centre, and Right inputs, and two output tubing assemblies—one output assembly each for the Open System and Closed System output. **The Blaze+ Tubing kit is required for each Blaze+ formulation using a NxGen cartridge.**

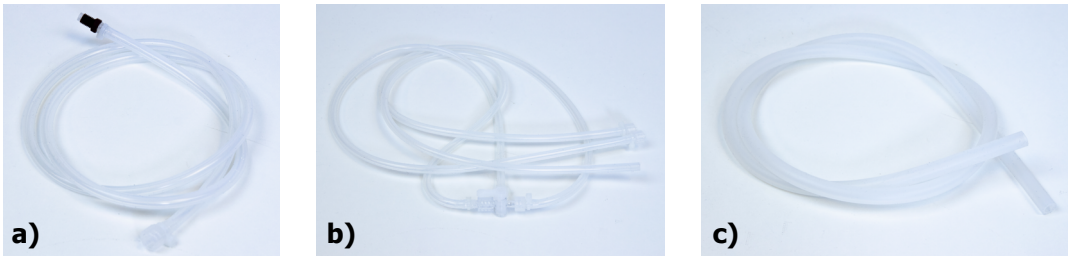


Figure 15-2: *The NanoAssemblr Blaze+ Tubing Kit contains a) three input tubes with threaded and Luer lock insert connections b) one output tubing set to connect directly to the NxGen Cartridge with a switch valve and Luer lock insert connections and c) tubing to connect to the output funnel and direct the fluid to an open vessel.*

KEY CONSIDERATION

Do not modify any tubing to the provided input tubing assemblies. The system has been calibrated to operate with the supplied tubing diameters and lengths to minimize hold-up volumes.

The total hold-up volume is approximately 15 mL for one channel on Blaze+. This volume must be included when preparing a sufficient amount of input reagents to prevent the channels from running dry during formulation.

3. **10L recipes can be created**
   The Blaze+ is capable of running Recipes with reagent volumes up to 10 litres and dilution volumes up to 30 litres for NxGen Cartridges. Blaze+ volumes are not available for Classic mixers.
4. **Fluid can be drawn in from external bioprocess bags**
   External bioprocess bags can be connected to accommodate large fluid volumes that are unable to fit in the Blaze+ instrument. If external bioprocess bags are used, the sippers should be disabled during Recipe creation and during a **Clean** if necessary. When using external bioprocess bags, the sippers do not need to lower to draw up fluid. To prevent this, **Lower Sippers** must be unchecked.

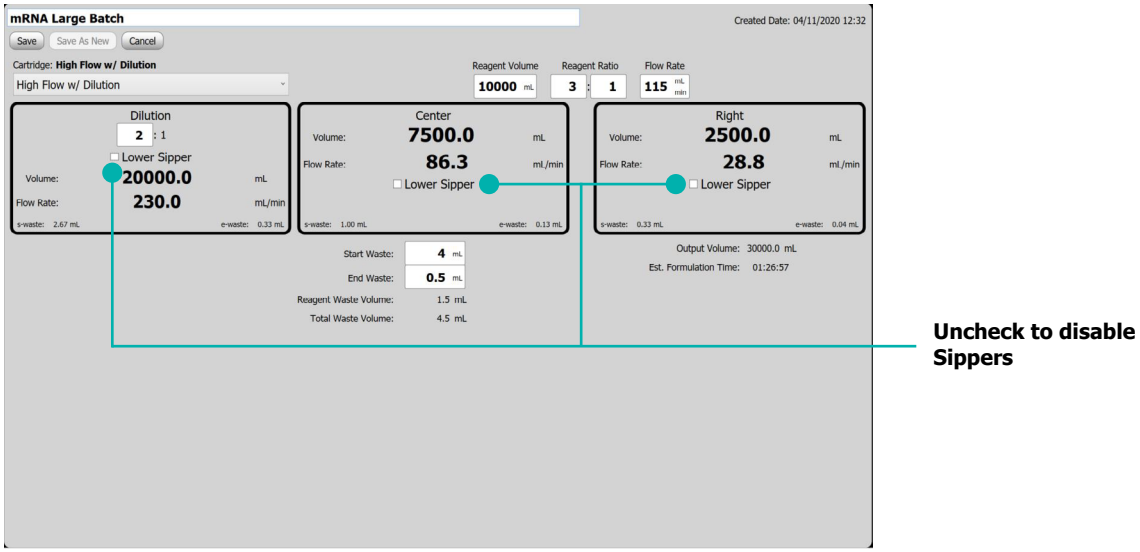


Figure 15-3: *Recipe Tab with Lower Sippers Unchecked*

KEY CONSIDERATION

A recipe can have different input options for different channels. If one of the input volumes is 1 L or less, it is acceptable to load the vessel under the sipper as described in Section 8.2 Input Vessels.

15.2 Setting Up Input and Output Vessels for the Blaze+

15.2.1 Input Vessels

Before creating a recipe for the formulation, it is necessary to determine how the input reagents will enter the Blaze+: either by external bioprocess bags, internal vessels or a mix of the two. Sippers should be disabled for external bioprocess bags.

15.2.1.1 Choosing Input Vessels and Tubing

The use of an internal or external vessel is determined by the amount of reagent being used for a formulation. An internal vessel and the sipper should be used for an input channel if the volume is 1 L or less.

If the input channel volume is greater than one liter, external bioprocess bags should be used. Use bags with tubing that has minimal volume between the bag port and the accompanying connection point, no greater than 3.5 mL. The Blaze+ is capable using a both internal and external input vessels during a single formulation if a formulation calls for volumes that are below and above one liter in the different input channels.

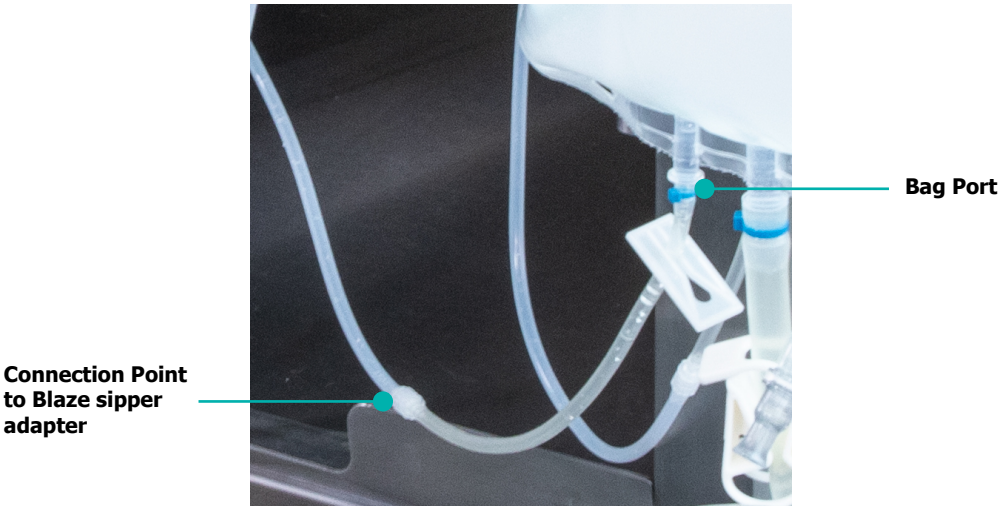


Figure 15-4: *Volume between the Bag Port and the Bag Connection*

The Blaze+ Tubing Kit has been designed to connect with Luer lock body connections. The following table displays recommended bags from Pall Life Sciences. These bags contain a tubing material (Platinum cured silicone) that offer better compatibility with organic solvents.

Size	Pall Life Sciences Part Number
2 L	650-202M
5 L	650-202P
10 L	650-202R

Table 16: *Recommended Pall Life Sciences Bio-Process Bags*

A list of recommended bags from Thermo Fisher Scientific are provided here. These bags contain tubing material (C-Flex™) that may not be compatible with aggressive organic solvents such as 100% ethanol.

Size	Thermo Fisher Scientific Part Number
2 L	SH30713.01
5 L	SH30713.01
10 L	SH30713.02
20 L	SH30713.03
50 L	SH30713.04

Table 17: *Recommended Thermo Fisher Scientific Bio-Process Bags*

It is strongly recommended that compatibility studies are performed with the bags being used to formulate with the Blaze+.

15.2.1.2 Positioning Input Vessels

If internal input vessels are being used with the Blaze+, they should be placed inside the instrument. The alignment features on the baseplate act as placement guides, but sipper alignment should always be confirmed through the Sipper tab prior to formulation, see Section 8.2.1 Centering Input Vessels.

If external input bags are being used with the Blaze+, consider the factors below:

1. **Air should not enter the Blaze fluid path during a run.** The external bags should be positioned so air bubbles will rise away from the output tubing. Bio-process bags should be hung vertically or should be placed on a surface that has a slant, maintaining the output tubes at the very bottom of the bags.
2. **External input bags cannot be further than 60 cm away from the sipper arms in the Blaze instrument.** Due to the length of the supplied input tubing, fluid management equipment such as bag stands, totes, and trolleys must be placed as close as possible to the Blaze+ instrument. The Blaze+ should have sufficient space around the instrument to allow for placement of external bags.

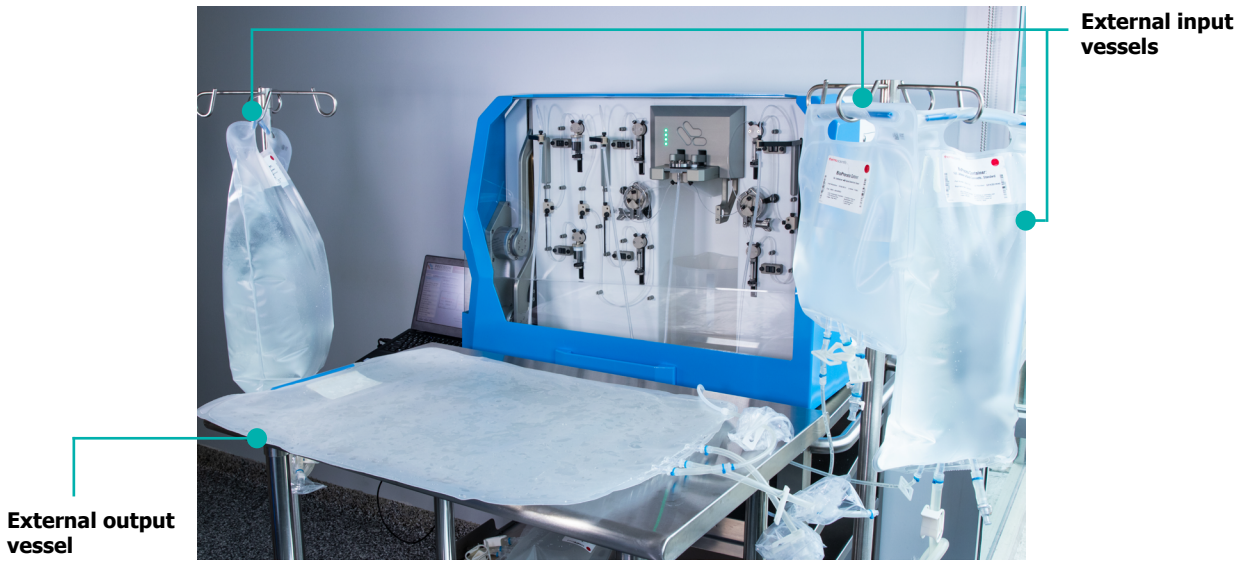


Figure 15-5: *Recommended Input Bag Layout*

15.2.1.3 Connecting Input Tubing

The supplied input tubing connects bags with Luer lock connections to the threaded fittings on the Blaze+. The input tubing assemblies may be connected before or after sanitization or washing of the Blaze fluid path – see Sections 15.3.1 Running a Clean on Blaze+ and 9.1 Initializing the Fluid Path on Blaze.

The steps involved in setting up the input tubing are outlined below.

1. Unscrew the sippers and store them in a safe place. Ensure the fittings do not fall off the sipper straw. See Section 13.4 Replacing Sippers.
2. Connect the threaded fitting on the input tubing assembly to the port on the sipper adaptor. Finger tighten the fitting.
3. Push the tubing into the tubing clip to secure it.
4. Connect the Luer lock insert connection to the bag, and open the valve on the bag tubing.

Figure 15-6: *Bio-process Bag Connection and Valve*

15.2.2 Output Tubing and Vessels

After the input vessels have been set up, the Blaze+ is ready for the the attachment of the output bags. The output options should be considered before creating a recipe as this option will affect the waste volumes that are used during Recipe creation.

15.2.2.1 Choosing Output Tubing and Vessels

The output formulation can be collected by connecting directly to the cartridge, or by setting up a funnel underneath the cartridge to collect the fluid. The advantages and limitations of each option are outlined in the table below.

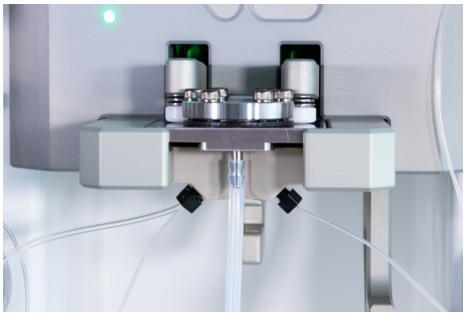
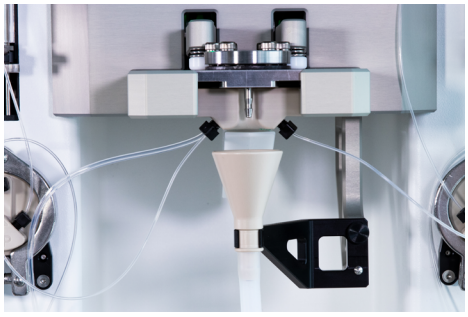
Closed System	Open System
Tubing connected directly to the Cartridge, directing fluid to an external closed vessel. 	Funnel with tubing attached, directing fluid to an external open vessel. 
Advantages Reduced risk of contamination with a closed fluid path.	**Advantages** Start and End Waste can be collected using the built-in automated Waste Collector. The collection vessel can directly feed downstream processes.
Limitations Waste can only be collected by manually switching a valve between the waste and sample output vessels. The valve in the fluid path introduces one additional material, Polycarbonate, to consider during chemical compatibility assessment. The collection vessel cannot directly feed downstream processes.	**Limitations** Cannot feed into an enclosed vessel as air will enter and prematurely fill the output vessel.

Table 18: *Comparison of Open and Closed System Advantages*

The open system utilizes the built-in waste collection mechanism on the Blaze+ to ensure particles are collected during steady state flow conditions and to minimize the amount of waste generated. **With the open system, the same waste volumes as a standard Blaze formulation should be used,** see Section 10.1.1 Determining Waste.

If the closed system is selected, both start and end waste volumes should be set to 0. The automated Waste Collector is not used in this case as the tubing connects directly to the cartridge, impeding the switching mechanism. Instead, switching can be manually performed by turning a switch valve on the tubing assembly between waste and the sample collection vessel.

The time between start and valve switching is determined by the flow rate being used in the Recipe. Once fluid has passed the valve on the Closed System output tubing, the predetermined time should pass before switching the valve.

To determine the time needed to switch, the target waste volume should be divided by the flow rate.

15.2.2.2 Positioning Output Vessels

There are two considerations when positioning the external output bags around the Blaze+ instrument.

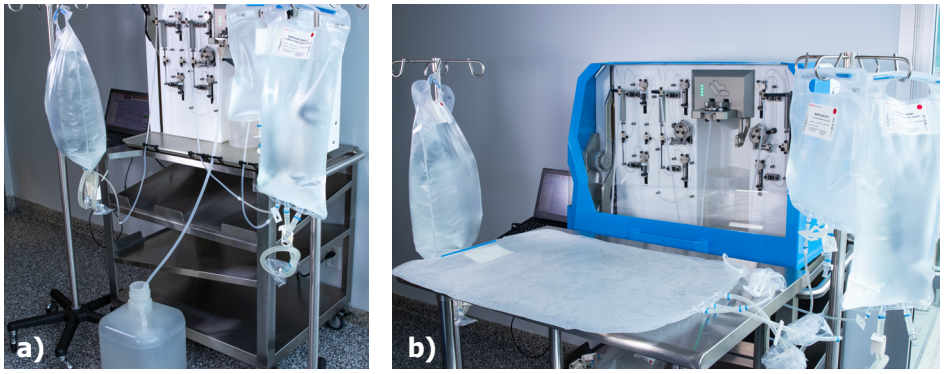


Figure 15-7: *Recommended Output Vessel Layout – a) Open System b) Closed System*

1. For both the Closed and Open output systems, **the output vessels or bags should be positioned below the Blaze+ cartridge.** Collecting the output fluid using the funnel option relies on gravity to ensure the fluid flows into the external bag. The output vessel should be below the Blaze instrument with **no kinks or restrictions in the tubing between the funnel and vessel.** If restrictions exist, fluid may not drain out of the output tubing correctly and spill out of the funnel.
2. The open system requires that **the funnel is set up to direct fluid to an open vessel only.** If the fluid is directed into an enclosed vessel such as a bio-process bag, air will be carried into the bag with the fluid and will remain in there, resulting in pre-mature filling of the output vessel.

Figure 15-8: *Swollen Bag When Using Open System*

Figure 15-9: *Open System Output Tubing into Open Vessel*

15.2.2.3 Connecting Output Tubing for a Closed System

The closed system allows formulations to be collected directly into a bio-process bag. The output tubing assembly connects the NxGen Cartridge and a bio-process bag. Sanitization and washing of the Blaze+ fluid path may be performed before and after connecting the output tubing– see Sections 15.3.1 Running a Clean on Blaze+ and 9.1 Initializing the Fluid Path on Blaze.

The setup steps for the closed system output tubing are outlined below.

1. Remove the output tubing assembly from the packaging. Confirm that the three tubes are connected to the switch valve. Two tubes should have Luer lock insert connections attached.
2. Remove the Waste Collector.
3. Connect the end without a fitting to the barb output on the cartridge. Push into place and secure with a cable tie if desired.
4. Push the tubing into the tubing clip on the baseplate edge to secure it.
5. With the Closed Output tubing, connect one Luer lock insert connection to the sample collection bio-process bag. Connect the other to the waste collection vessel.

KEY CONSIDERATION

The output collection bag and the waste collection may be connected to either Luer connection of the output tubing assembly, but the ports in which they connect determine how the valve should be turned to collect waste.

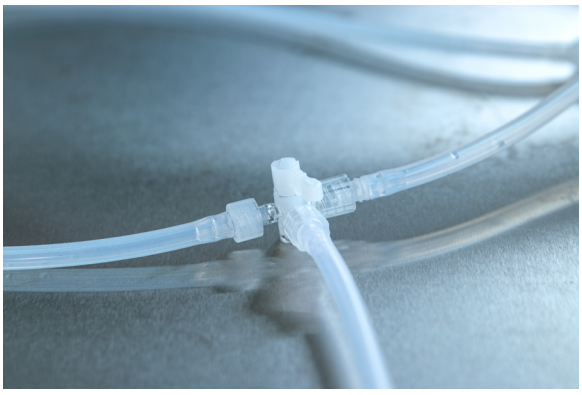


Figure 15-10: *Closed System Tubing*

6. Open the valves on the bags if they are present.
7. Ensure the switch valve is switched to divert fluid to the waste container.

KEY CONSIDERATION

NOTE that the direction of the divertor indicates the line that is turned OFF.

15.2.2.4 Connecting Output Tubing for an Open System

The open system output tubing connects the barb output on the funnel to an open external bag. Sanitization and washing of the Blaze+ fluid path may be performed before and after connecting the output tubing – see Sections 15.3.1 Running a Clean on Blaze+ and 9.1 Initializing the Fluid Path on Blaze.

The steps to set up the open system output tubing are outlined below.

1. Remove the tubing from the packaging.

Figure 15-11: *Open System Output Tubing*

2. Secure the funnel holder on the instrument and clip in the Blaze+ funnel with the barb feature.
3. Ensure the Waste Collector is in place.
4. Attach one end of the tubing to the barb on the funnel. Push into place and secure with a cable tie if desired.
5. Insert the other end of the tubing into the collection vessel. Ensure this vessel is open to allow any air to escape.

15.3 Performing Recipe Actions on the Blaze+

15.3.1 Running a Clean on the Blaze+

Like the standard Blaze, the **Clean** recipe action draws in cleaning reagents and pushes the fluid through the entire fluid path, including the cartridge, then into the waste container. A new, previously used, or cleaning cartridge may be used for running a **Clean**.

Like the Blaze fluid path, the Blaze+ Tubing Kit is not sterile; therefore, building the additional Blaze+ tubing into the sanitization and cleaning protocol should be considered. Depending on the level of cleanliness required for the formulation, cleaning or sanitization protocol may be performed with or without the Blaze+ Tubing Kit attached.

If the Blaze+ Tubing Kit is included during the cleaning step, ensure the Lowering Sippers are unchecked on the *Clean* tab before proceeding. See Section 5 Workflow for more information about **Clean**.

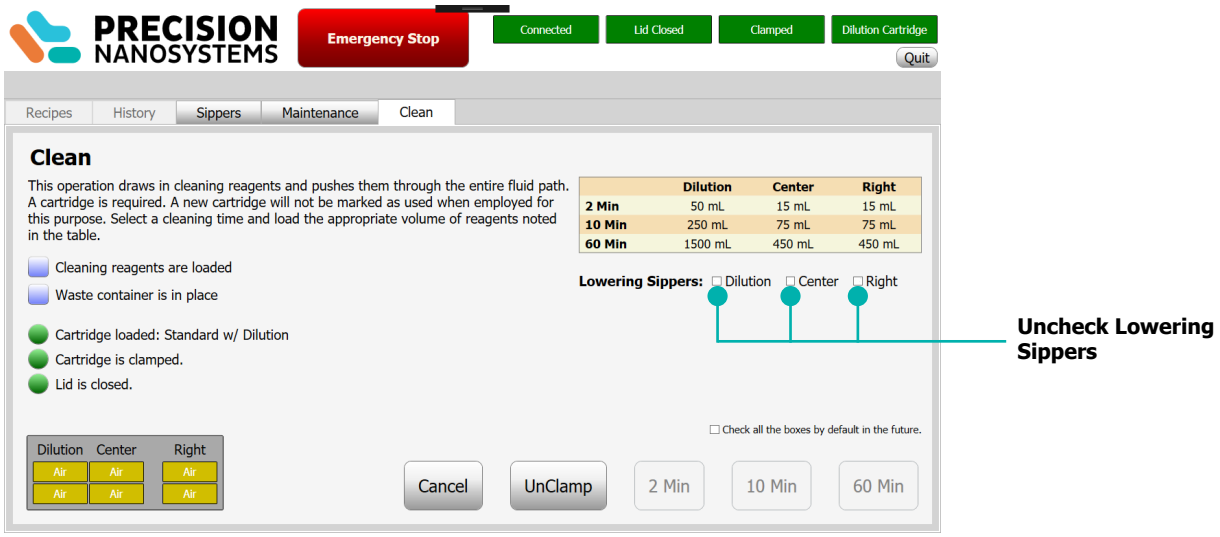


Figure 15-12: *Clean Tab with Lowering Sippers Unchecked*

15.3.2 Running a Recover on Blaze+

Like the standard Blaze, a **Recover** empties the fluid path by pumping the contents backwards and out through the sippers. The recovered fluid may be kept. The input tubing should be disconnected for a **Recover** – otherwise fluid will be pumped into the input bags. See Section 5 Workflow for more information about **Recover**.

When running a **Recover**:

1. Ensure the cartridge is removed.
2. Ensure the input tubing is disconnected from the Sipper adaptors.
3. Ensure the Sippers are attached.
4. Ensure waste collection vessels are positioned beneath the Sippers.
5. Ensure the Waste Collector is attached.

15.3.3 Running a Purge on Blaze+

Like the standard Blaze, a **Purge** voids the tubing assembly by replacing the fluid with air. The fluid is pumped through the cartridge and into the waste container. For the Blaze+, the input and output tubing should be disconnected– otherwise fluid will be drawn in from the input bags and pumped into the output bags. See Section 5 Workflow for more information about **Purge**.

When running a **Purge**:

1. Ensure a cartridge is installed. A new, previously used, or cleaning cartridge may be installed.
2. Ensure the input tubing is disconnected from the Sipper adaptors. It is optional to connect the Sippers.
3. If using the funnel, ensure the Waste Collector is attached and that a waste vessel is underneath the Waste Collector.
4. If using the closed system output tubing, ensure the valve is set to waste, or remove the tubing and attach the Waste Collector.

15.4 Removing Input Tubing Assemblies from Blaze+

When removing tubing assemblies following a **Clean** or **Formulate**, the tubing may contain residual fluid. Gloves and other personal protective equipment should be used.

To remove the assemblies:

1. Close the valve output on the bio-process bag tubing.
2. Disconnect the Luer connection at the output of the bag.
3. Remove the tubing from the tubing clip at the base of the Blaze
4. Disconnect the threaded fitting at the sipper adaptor.
5. Reconnect the Sippers or another external input.

15.5 Additional Steps for the Blaze+ Protocol

The Blaze+ operates similarly to the standard Blaze, but additional steps and checks are essential to ensure a successful formulation due to the additional tubing, fittings and instrument set up. These additional steps are necessary to incorporate into the operation of the Blaze+ to avoid a formulation failure.

Prior to formulating large batches with valuable reagents, a practice run using water or inexpensive reagents is recommended to become familiar with the Blaze+ and its features. Developing a step-by-step procedure tailored to the formulation requirements is strongly recommended.

A starting point for developing a suitable procedure is found in the sections below.

15.5.1 Added Steps to the Setting Up the Blaze+

Setting up the Blaze+ requires additional steps during recipe creation, and input and output set up to ensure proper operation. These additional steps below should be added to the formulation procedure if necessary. See Sections 15.2 Setting Up Inputand Output Vessels for Blaze+, Section 8 Preparing the Instrument and Section 10.1 Creating a Recipe.

Recipe Creation

- ☐ Input Reagent Volume, Reagent Ratio and Reagent Flow Rate in the Recipe Tab
- ☐ Select Cartridge High Flow or High Flow w/ Dilution
- ☐ If High Flow w/ Dilution has been selected, enter the Dilution Ratio
- ☐ For channels with an input greater than one litre (i.e. using external input vessels), uncheck Lower Sippers
- ☐ Input Start and End Waste
- ☐ If using the closed output, set both waste values to 0 mL as the automated waste collection is being bypassed.
- ☐ If using the open system, reference Section 10.1.1 Determine Waste.

Attaching Input Tubing

- ☐ Remove the sipper from the channels that will use external input connections.
- ☐ Remove the tubing from the packaging.
- ☐ Screw the threaded fitting on the tubing assembly into the sipper adaptor.

While holding the sipper adaptor, finger tighten the threaded fittings.

- ☐ Secure the tubing in the tube clips.
- ☐ Connect the Luer connections to the bio-process bags.
- ☐ Ensure the valves are open once the connection to the external vessel is made.

Attaching Output Tubing

Closed System

- ☐ Insert the NxGen Cartridge.
- ☐ Remove the Waste Collector from the instrument.
- ☐ Remove the Closed System tubing from the packaging.
- ☐ Connect the end with no fitting to the barb output on the cartridge. Secure with a cable tie if necessary.
- ☐ Secure the tubing in the tube clips.
- ☐ Connect one Luer fitting to the sample collection bio-process bag beneath the instrument.
- ☐ Ensure valves on the bag tubing are open.
- ☐ Connect the other Luer fitting from the cartridge tubing to a waste bag or position it into the waste collection vessel.
- ☐ If present, ensure any valves on the waste vessel are open.
- ☐ Turn the stopcock valve to divert to waste. The diverter (displaying OFF) should point to the sample collection bag.
- ☐ By taking the flow rate and ratios into account, determine the amount of start fluid to divert to the waste vessel. See Section 15.2.2.1 Choosing Output Tubing and Vessels.

Open System

- ☐ Insert the NxGen Cartridge.
- ☐ Install the Waste Collector if not attached.
- ☐ Ensure that the Waste Collection Vessel is positioned under the collector.
- ☐ Connect the Blaze+ funnel to the instrument.
- ☐ Remove the Open System tubing from the packaging.
- ☐ Connect the Open System tubing to the barb connector on the funnel.
- ☐ Secure the tubing in the tube clips.
- ☐ Position the other end of the tubing in the external collection vessel. Ensure the vessel is not sealed so air can escape as it fills.

15.5.2 Additional Steps to Performing Recipe Actions on the Blaze+

Performing recipe actions and formulations on the Blaze+ requires additional checks and steps to ensure successful formulation. These additional steps below should be added to the formulation protocol if necessary. See Section 15.3 Performing Recipe Actions on Blaze+, Section 5 Workflow, and Section 10.3 Running a Formulation.

Running a Clean

- ☐ Ensure a cartridge is installed. A new, previously used, or cleaning cartridge may be installed.
- ☐ If using external vessels during a **Clean**, check that all input and output external vessels are attached. If not using external vessels, ensure the sippers are attached and that the cleaning fluids are loaded underneath them.
- ☐ If applicable, ensure the valves on the bag tubing are open.
- ☐ If using the funnel, ensure the Waste Collector is attached and a vessel is positioned under the collector.
- ☐ If using the Closed output system, ensure the valve is turned such that the fluid is diverted to the tubing being cleaned.
- ☐ Click **Clean** on the Blaze software.
- ☐ On the Clean tab, uncheck **Lowering Sippers** for the channels that have the Blaze+ tubing assemblies attached.
- ☐ Confirm each step on the screen and then select either the 2-, 10- or 60-minute options.

Running a Recover

- ☐ Ensure no cartridge is installed.
- ☐ Ensure the input tubing is disconnected from the Sipper adaptors.
- ☐ Ensure the sippers are attached
- ☐ Ensure waste collecting vessels are positioned beneath the Sippers.
- ☐ Ensure the Waste Collector is attached.

Running a Purge

- ☐ Ensure a cartridge is installed. A new, previously used, or cleaning cartridge may be installed.
- ☐ Ensure the input tubing is disconnected from the Sipper adaptors. It is optional to connect the Sippers.
- ☐ If using the funnel, ensure the Waste Collector is attached and a vessel or pitcher is positioned under the collector.

Running a Formulate

- ☐ Ensure that there is no fluid in the Blaze+ input tubing.
- ☐ Check that all input and output external vessels are attached.
- ☐ Open the valves on the bag tubing connected to the Blaze+ input and output tubing. Some fluid may enter the Blaze+ tubing assembly at this stage.
- ☐ If using the funnel, ensure the Waste Collector is attached and a vessel is positioned under the collector.
- ☐ Click formulate, confirming each step on the formulation screen.
- ☐ If using the closed system output option, switch the stopcock valve diverter after the determined amount of time. If using the open system output option, the built-in automated waste collector will switch from sample to waste.

16 Regulatory

A급 기기 (업무용 방송통신기자재)
이 기기는 업무용(A급)으로 전자파적합기기로서
판매자 또는 사용자는 이 점을 주의하시기 바라며,
가정 외의 지역에서 사용하는 것을 목적으로 합니다.

Translation: Class A (Broadcasting and Communication equipment for Business)
Sellers and users should note that this equipment is an electromagnetic device for business (class A), and is intended for use outside the home.
Power supply needs to be certified (KC).

取得審驗證明之低功率射頻器材,非經核准,公司、商號或使用者均不得擅自變更頻率、加大功率或變更原設計之特性及功能。低功率射頻器材之使用不得影響飛航安全及干擾合法通信;經發現有干擾現象時,應立即停用,並改善至無干擾時方得繼續使用。前述合法通信,指依電信管理法規定作業之無線電通信。低功率射頻器材須忍受合法通信或工業、科學及醫療用電波輻射性電機設備之干擾

Translation: NCC Warning Statement

Article 12
Without permission, any company, firm or user shall not alter the frequency, increase the power, or change the characteristics and functions of the original design of the certified lower power frequency electric machinery.

Article 14
The application of low power frequency electric machineries shall not affect the navigation safety nor interfere a legal communication, if an interference is found, the service will be suspended until improvement is made and the interference no longer exists.

17 Ordering Information

To place orders for the the NanoAssemblr Blaze, contact your Field Application Scientist or local Precision Nanosystems consultant.

Instrument:

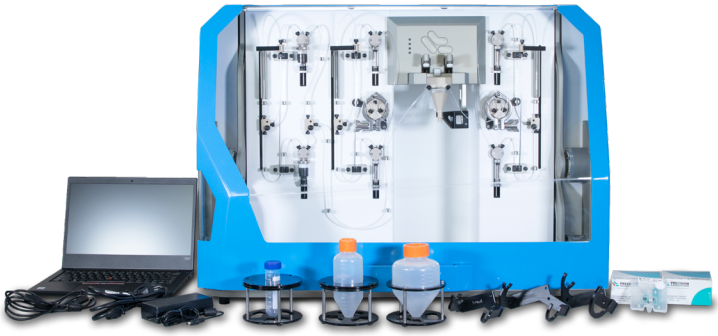
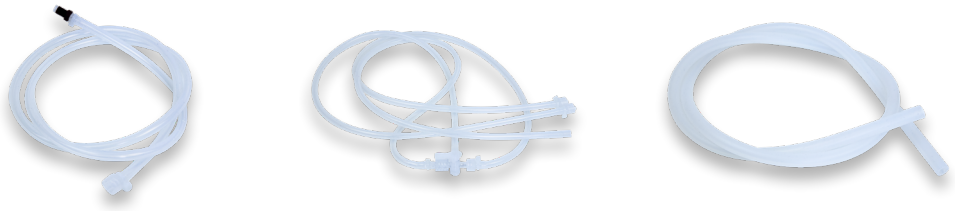
Image		
		
Name	**Product Code**	**Includes**
NanoAssemblr Blaze™	NIB0055	NanoAssemblr Blaze instrument Control laptop and USB cable Power supply and power cable Input and output vessel holders Spare sippers NanoAssemblr Blaze Cleaning cartridge Various reagent and sample bottles One year warranty
Image		
		
Name	**Product Code**	**Includes**
NanoAssemblr Blaze+ Upgrade	NIB0061	NanoAssemblr Blaze+ Adjustment Plate 4 Tube clips 2 Safety clips 1 Funnel Adapter 1 Open System funnel

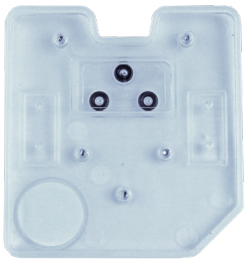
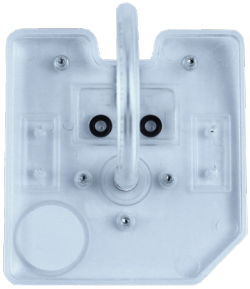
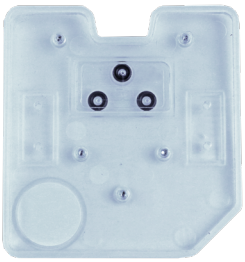
Image		
		
Name	**Product Code**	**Includes**
NanoAssemblr Blaze+ Tubing Kit	1000535	Open System Output Tubing Closed System Output Tubing 3x Input Vessel Tubing

Service:

Name	Product Code	Includes
NanoAssemblr Blaze Total Service Plan	NOB0004	Prepaid yearly service product including prioritized technical assistance, defect repairs (including spare parts), and one preventative maintenance per year. Shipping and loaner instrument (if needed) included.
NanoAssemblr Blaze+ System Service Package	1000556	Prepaid yearly service product including one onsite service and two preventative maintenance services per year.

Cartridges:

Image	Product Code	Includes/Description
	1000458	1x NxGen 400™ Blaze Cartridge without dilution
	1000459	1x NxGen 400D™ Blaze Cartridge with in-line dilution

	1000460	1x NxGen 500™ Blaze Cartridge without dilution
	1000461	1x NxGen 500D™ Blaze Cartridge with in-line dilution
	NIB0002	1x NanoAssemblr Classic Blaze Cartridge - No Dilution
	NIB0003	1x NanoAssemblr Classic Blaze Cartridge - In-line Dilution
	NIB0009	1x NanoAssemblr Classic Blaze Cartridge - Cleaning

Your Field Application Scientist's contact information:

To learn more about the NanoAssemblr Blaze™, visit precisionnanosystems.com/our-technology/blaze

Or to learn more about the NanoAssemblr Platform, visit precisionnanosystems.com/systems/

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PNI is a global leader in ushering in the next wave of genetic medicines in infectious diseases, cancer and rare diseases. We work with the world's leading drug researchers to understand disease and create the therapeutics and vaccines that will define the future of medicine. PNI offers proprietary technology platforms and comprehensive expertise to enable researchers to translate disease biology insights into non-viral genetic medicines.

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