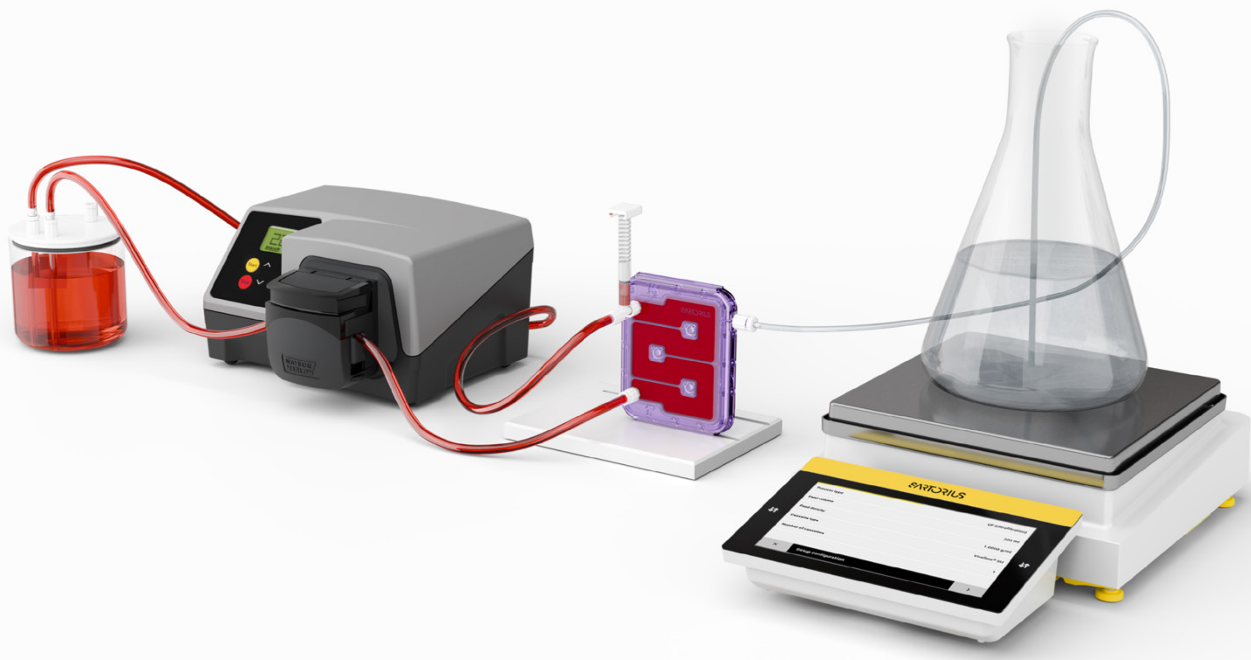
Product Guide

Vivaflow® System

Lab Scale Tangential Flow Filtration with the Vivaflow® QApp



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1. Purpose

This guide provides recommended procedures to configure and run automated lab scale tangential flow filtration (TFF) processes on Vivaflow® System. This guide is not a replacement for the hardware and consumable operating instructions, which must be read and understood in full before using the system.

2. Descriptions

2.1 Vivaflow® Consumables

Figure 1 shows Vivaflow® SU and Vivaflow® consumables. These lab scale TFF cassettes are recommended for ultrafiltration (UF) and/or diafiltration (DF) of feed volumes between 100 mL and 5 liters, and are supplied with tubing kits for UF. Additional tubing for DF, and replacement tubing kits for the multi-use cassettes can be purchased separately.

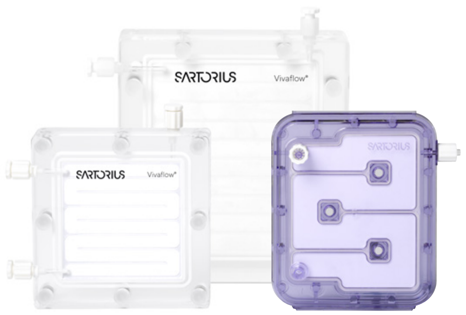

Figure 1: Vivaflow® SU and Vivaflow® TFF cassettes.

2.2 Vivaflow® System

Vivaflow® System consists of all hardware and software needed to automate TFF processes on Vivaflow® consumables. The core hardware components are a Watson-Marlow 323 series pump, Cubis® III precision balance and RS232 data cable, which are optionally supplied with a feed reservoir and cassette stand (Figure 2). The software is the Vivaflow® QApp, which is factory licensed and pre-installed on the balance as part of the standard software package.

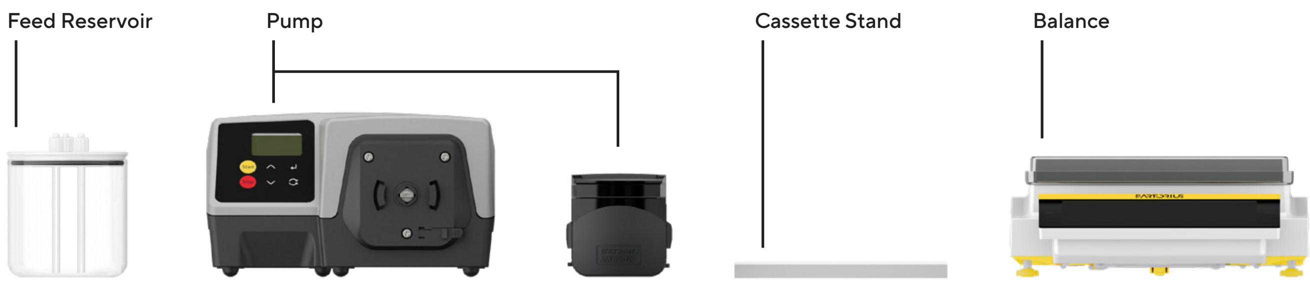

Figure 2: Vivaflow® System hardware includes a pump and balance, which are connected via data cable (not shown). Optional accessories include a feed reservoir and cassette stand.

3. System Set Up

3.1 Hardware and Consumables

Vivaflow® System supports several TFF process modes, each comprising one or more process steps (see **4.3.1 Process Settings, Table 3**). Cassette prewashing and retentate collection are optional steps that can be included in any process mode. Figure 3 shows how the consumables and hardware should be set up for each type of process step.

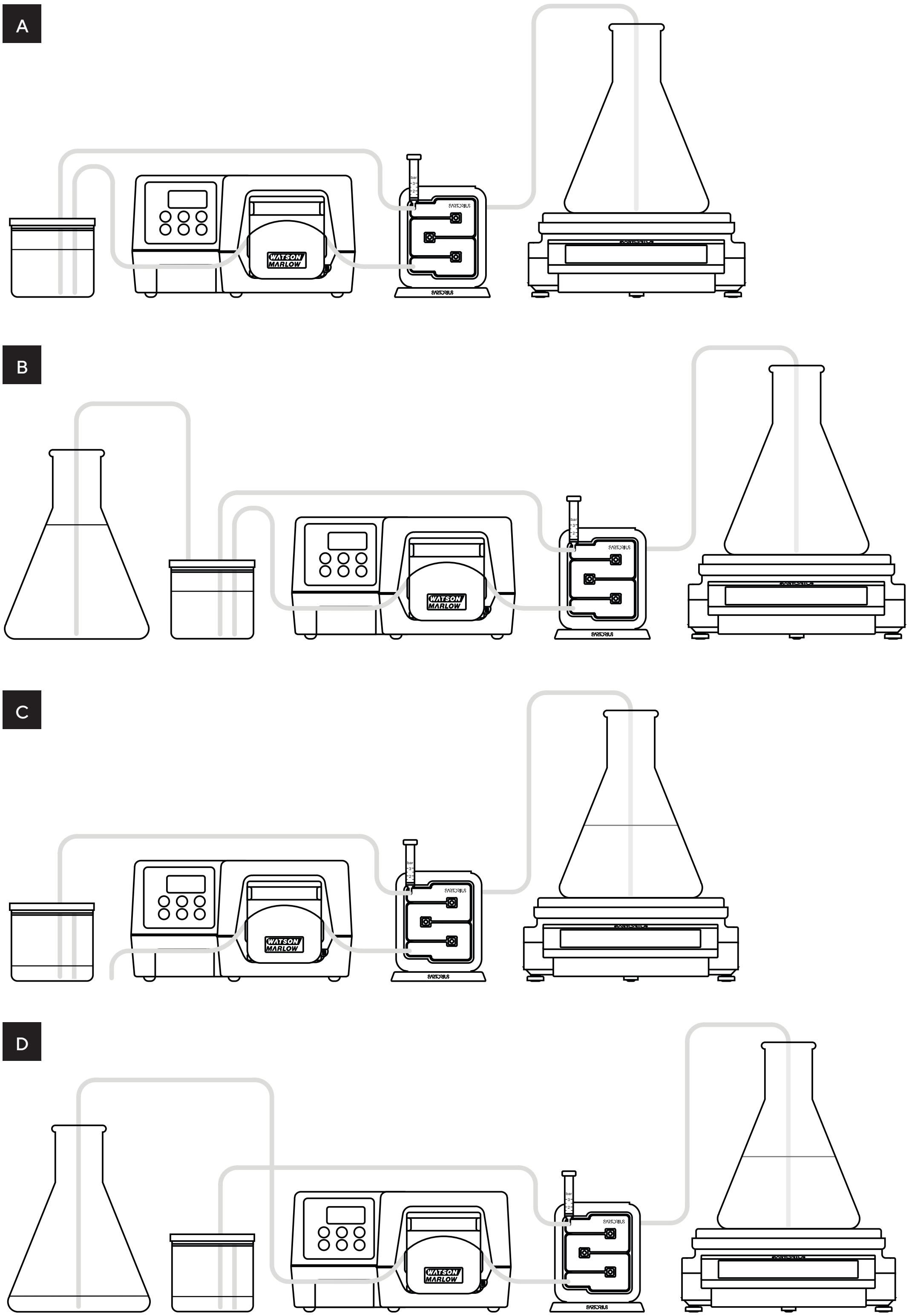

Figure 3: Consumable and hardware set up for prewashing or ultrafiltration (A), diafiltration (B), and retentate collection by air purge (C) or buffer flush (D).

For TFF process automation, the pump and balance communicate via the RS232 data cable.

To configure the RS232 interface on the pump:

1. Connect one end of the cable to the 9-way D connector on the balance (Figure 4A)
2. Connect the other end of the cable to the 9-way D connector on the pump (Figure 4B)

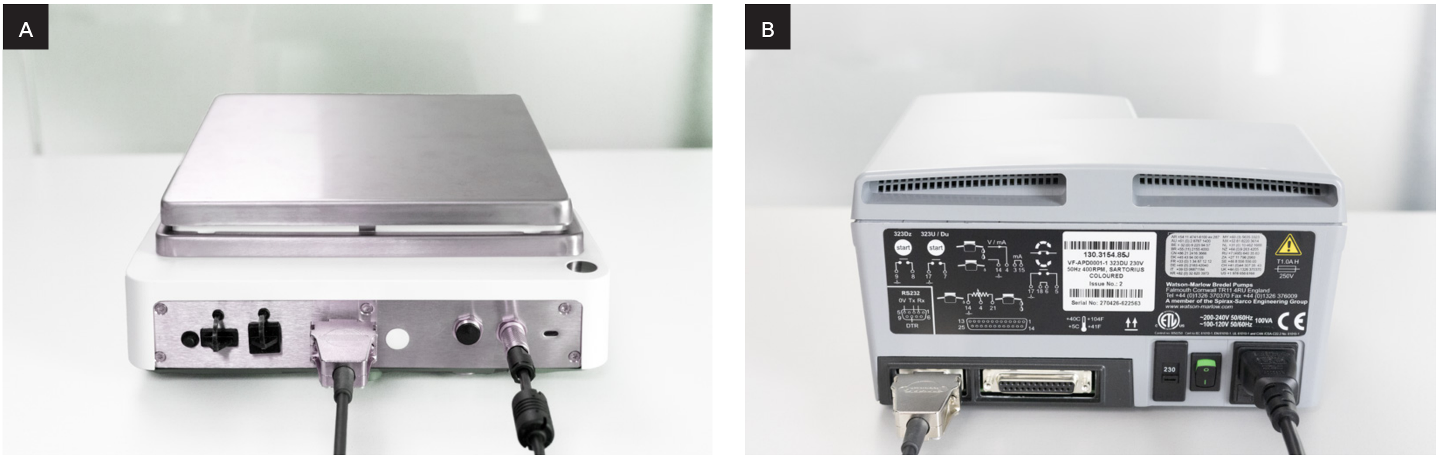

Figure 4: RS232 data cable connection to the balance (A) and pump (B).

3.2 Hardware Interface

3.2.1 Pump Settings

To configure the RS232 interface on the pump:

1. Press the **MODE [↵]** key until "dig" is shown on the display (Figure 5)

Figure 5:Pump display showing the pump in digital mode.

The pump does not save or recall the last mode that was used. Therefore, this procedure must be repeated each time the pump is switched on.

3.2.2 Balance Settings

To configure the RS232 interface on the balance:

1. Open the **SETTINGS** menu [🔧]
2. Press **Connections** → **Interfaces** → **COM-RS232 interface**
3. Press **EDIT** [✎]
4. Press each setting name, select the value listed in Table 1, and press **OK** [✓]
5. Press **OK** [✓] to save the configuration

Setting Name	Value
Protocol	QAPP direct
Log communication	Off
Activation at	Device startup
Baud rate	9600 baud
Data bits	8 data bits
Stop bits	2 stop bit(s)
Parity	None
Handshake	None

Table 1: RS232 interface configuration required for the balance to control the pump.

The configuration is saved and recalled by the balance, unless the settings are changed for another application. Therefore, reconfiguration of the RS232 interface on the balance is not normally required.

4. TFF Process Set Up

4.1 Starting the Software

To start the software on the balance:

1. Press the **Vivaflow®** task element (Figure 6)

Figure 6: Vivaflow® task on the main menu of the balance.

The main menu in the software lists two options – one for pump configuration and one for process configuration (Figure 7).

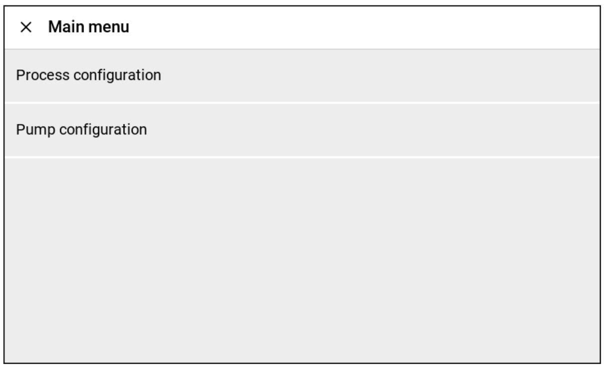

Figure 7: Vivaflow® QApp main menu.

4.2 Pump Configuration

The pump configuration defines the pump model and pump speed settings. For most applications, the default configuration (Table 2) does not need to be changed.

Setting Name	Value
Pump model	Watson-Marlow 323 Pump
Priming speed	100 rpm
Prewashing speed	100 rpm
High process speed	300 rpm
Low process speed	250 rpm
Process slow down speed	200 rpm
Retentate collection speed	300 rpm

Table 2: Pump configuration setting names and values

4.3 Process Configuration

The process configuration menu is used to define TFF process settings in two parts – setup configuration and target configuration.

To minimize set up steps, default values have been assigned to some settings in the process configuration menus, based on recommended operating conditions. Default values can be changed if required.

The process configuration menus adapt automatically in response to previous selections and the consumable or hardware operating limits. Therefore, some settings and values are not always displayed, or the value entered for a setting must be within an acceptable range. Settings with a missing or unacceptable value are displayed in red text. In addition, if a combination of setting values results in a target permeate mass that is too low or too high, an “invalid process configuration” message will be displayed. It is not possible to start a TFF process until all required settings have been assigned acceptable values.

4.3.1 Setup Configuration

The setup configuration menu is used to select the process mode and speed, and to define the sample and consumables that will be used (Figure 8). Setting names, definitions and possible values are listed in Table 3.

Figure 8: Setup configuration menu.

Setting Name	Definition	Possible Values
Process mode	The step(s) that should run in the TFF process	UF DF UF/DF DF/UF UF/DF/UF
Process speed	An arbitrary indication of the pump speed during UF and/or DF process steps	**High** Low
Feed volume	The volume of the starting sample, in units of milliliters (mL)	5 - 8,200 mL
Feed density	The density of the starting sample, in units of grams per milliliter (g/mL)	0.5 – 15 g/mL (**1 g/mL**)
Exchange buffer density	The density of the buffer that will be used in DF process steps, in units of grams per milliliter (g/mL)	0.5 – 15 g/mL (**1 g/mL**)
Cassette type	The name of the Vivaflow® cassette(s) that will be used	Vivaflow® SU Vivaflow® 50R Vivaflow® 200
Membrane type	The molecular weight cutoff and chemistry of the membrane in the Vivaflow® cassette(s) that will be used	2/5/10/30/100/300 kDa RC 3/5/10/30/50/100/300/1000 kDa PES 0.2 µm PES
Number of cassettes	The number of Vivaflow® cassettes that will be used	1 2

Table 3: Setup configuration setting names, definitions and possible values. Default values are shown in **bold.**

To define the setup configuration:

1. Press a setting name
2. Select or enter the required value and press **OK** [✓]
3. Repeat steps 1-2 for each setting
4. Press **NEXT** [›] to confirm the setup configuration settings

Defining the feed and exchange buffer densities can increase endpoint accuracy. However, if the densities are not known or can be assumed to be like that of water, the default values do not need to be changed.

4.3.2 Target Configuration

The target configuration menu is used to select whether a prewashing step should be performed before TFF, and to define the required endpoint for each process step (Figure 9). Setting names, definitions and possible values are listed in Table 4.

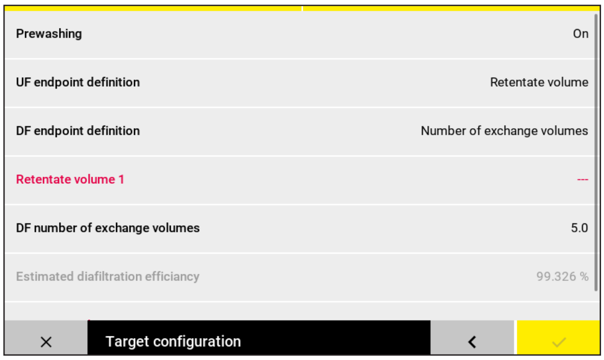

Figure 9: Target configuration menu, as displayed for the UF/DF/UF process mode.

Setting Name	Definition	Possible Values
Prewashing	An optional process step to prewash the cassette(s) with water or buffer before applying the feed sample	**On** Off
UF endpoint definition	How the end of a UF process step should be targeted	**Retentate volume** Concentration factor
UF concentration factor (1/2)	A multiplier representing the volumetric concentration factor that should be reached at the end of a UF process step	1.1 - 400
UF retentate volume (1/2)	The volume that the retentate sample should reach by the end of a UF process step, in units of milliliters (mL)	5 - 8,200 mL
DF endpoint definition	How the end of a DF process step should be targeted	**Number of exchange volumes** Theoretical exchange efficiency
DF number of exchange volumes	A multiplier representing the volume of exchange buffer relative to the feed volume that should be added to the flow path during a DF process step	1 - 50 (**5**)
DF theoretical exchange efficiency	A percentage representing the proportion of buffer, salt or low molecular weight impurities that should be removed from the feed during a DF process step	63.212 - 99.999 % (**99.326 %**)

Table 4: Target configuration setting names, definitions and possible values. Default values are shown in **bold**.

In UF process steps, concentration factors are solely based on sample volumes. These volumetric concentration factors may differ from molecular concentration factors, due to sample, membrane and process conditions. Therefore, if required, molecular concentration factors must be determined after TFF, by comparing appropriate measurements in retained samples of the feed and retentate.

In DF process steps that use a target number of exchange volumes, the default value is "5". This means that an amount of exchange buffer equivalent to five times the feed volume will be added to the flow path. For example, if the feed volume is 100 mL, then 500 mL of exchange buffer will be added. Using five exchange volumes will typically result in a buffer exchange or desalting efficiency >99%.

Alternatively, DF process steps can be configured with a target exchange efficiency. The software will calculate the number of exchange volumes needed to theoretically achieve the required efficiency (see **7.4 Permeate Mass for an Exchange Efficiency** for the calculations that are used). Table 5 lists the theoretical exchange efficiency for different numbers of exchange volumes. If required, actual exchange efficiency must be determined after TFF, by comparing appropriate measurements in retained samples of the feed and retentate.

Number of Exchange Volumes	Theoretical Exchange Efficiency
1	63.21 %
2	86.47 %
3	95.02 %
4	98.17 %
5	99.33 %
6	99.75 %
7	99.91 %
8	99.97 %
9	99.99 %
10	99.995 %

Table 5: Examples of the theoretical exchange efficiency for different numbers of exchange volumes.

To define the target configuration:
1. Press a setting name
2. Select or enter the required value and press **OK** [✓]
3. Repeat steps 1-2 for each setting
4. Press **OK** [✓] to confirm the target configuration settings

5. Running a TFF Process

5.1 Starting a TFF Process

To start a TFF process:

1. Follow the on-screen instructions to set up the flow path
2. Press **OK** [✓] to start the process

For flow path diagrams, see **3.1 Hardware and Consumables, Figure 3.**

Each TFF process begins with a priming step to remove air from the flow path. This is followed by prewashing (if selected) and the required process step(s).

5.2 Flow Path Adjustments

At the end of some process steps, the pump will stop automatically, and the software will provide instructions to adjust the flow path before the next step can be started (Figure 10). These instructions also list relevant minimum volumes and capacities. For example, the capacity required for the permeate vessel is given to prevent overfilling.

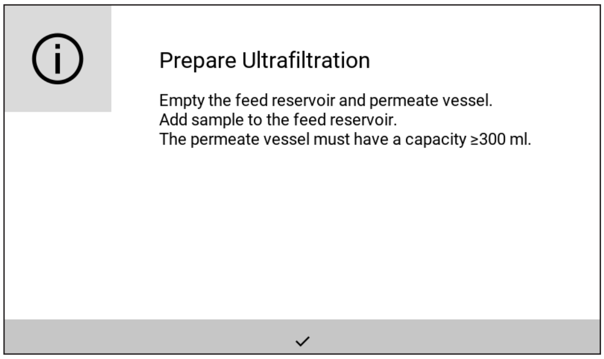

Figure 10: Example of flow path adjustment instructions.

When prompted at the end of a process step:

1. Follow the on-screen instructions to adjust the flow path
1. Press **OK** [✓] to start the next process step

For flow path diagrams, see **3.1 Hardware and Consumables, Figure 3**.

5.3 Process Adjustments

When a process is running, it is possible to adjust the pump speed, pause or stop the process, or skip to the next process step (Figure 11).

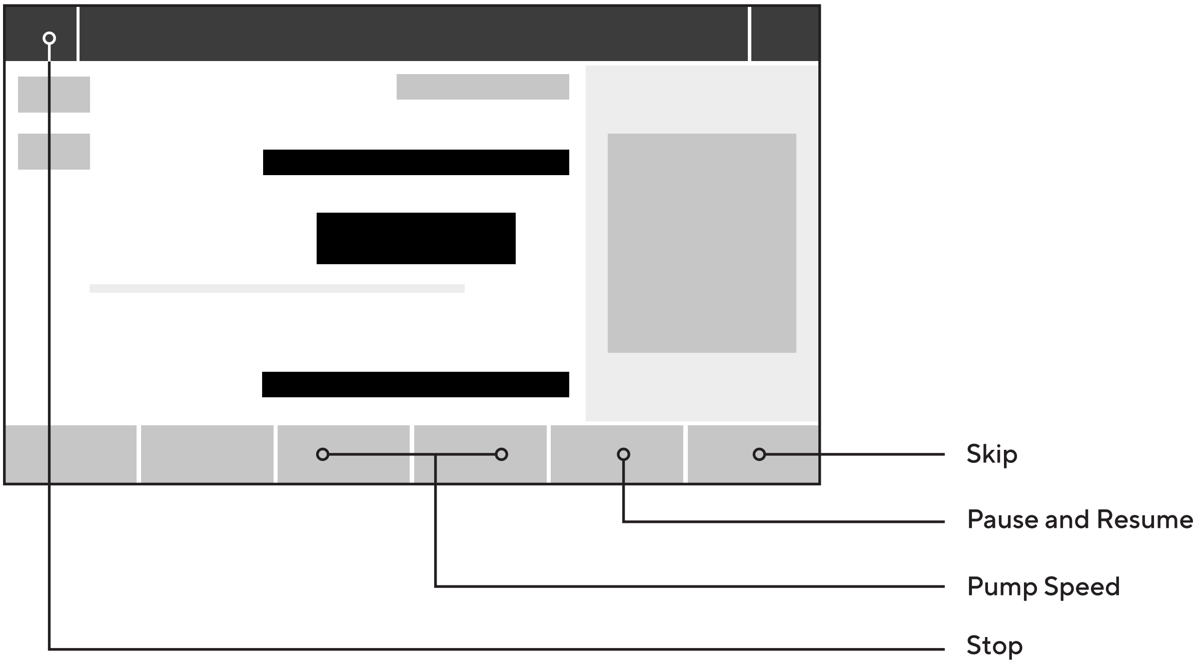

Figure 11: Controls for pump speed adjustment, pausing or stopping a process, and skipping to the next process step.

To adjust the pump speed:

1. Press **MINUS** [**-**] or **PLUS** [**+**] to decrease or increase in 1 rpm increments, or
2. Press-and-hold **MINUS** [**-**] or **PLUS** [**+**] to decrease or increase in 10 rpm increments

To temporarily interrupt a process:

1. Press **PAUSE**
2. To resume the process, press **RESUME**

To permanently interrupt a process:

1. Press **SKIP** [**>|**] to move to the next process step, or
2. Press **MENU** [⁝⁝⁝] to stop the entire process

When the balance is connected to a local area network and remote access is enabled, it is possible to make process adjustments via a supported web browser.

5.4 Monitoring TFF

The name and status of the current process step are displayed on the balance (Figure 12). In addition, color-coded graphics provide a visual representation of the process step that is running (Figure 13).

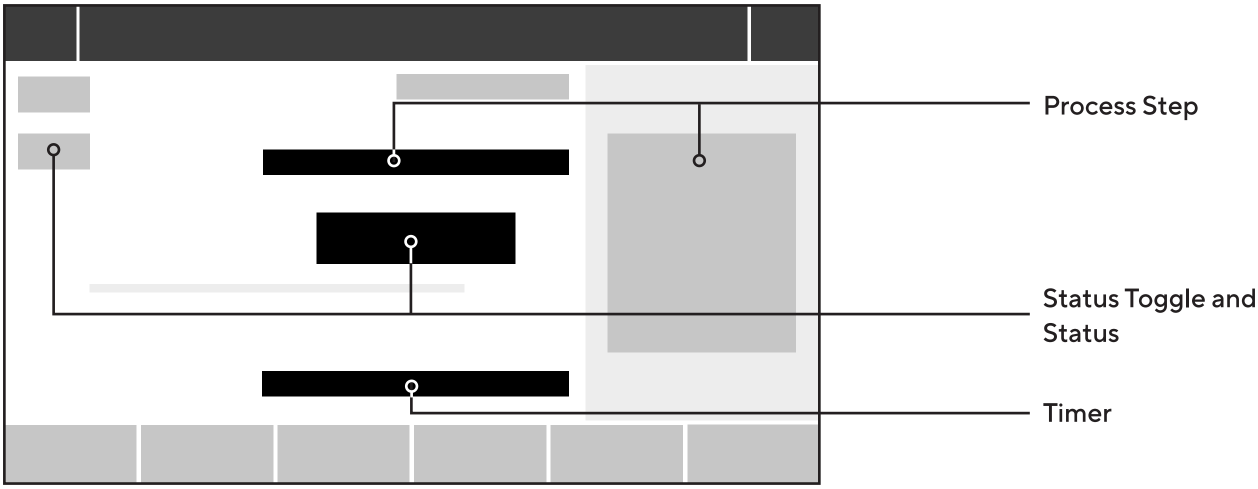

Figure 12: Current process step and status indicators include the step name and corresponding graphic. By default, the status of the current process step is displayed as a percentage complete. This can be changed to show current permeate volume by pressing the status toggle. In addition, a timer shows elapsed time or a countdown timer (for priming, prewashing and retentate collection steps) or estimated time remaining (for ultrafiltration and diafiltration steps).

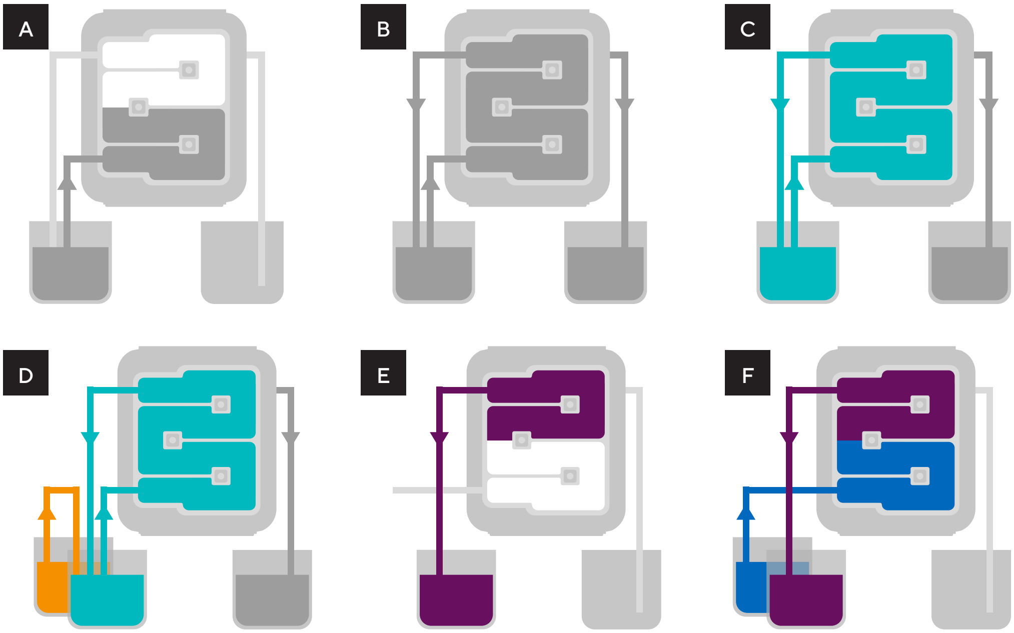

Figure 13: Graphics representing priming (A), prewashing (B), ultrafiltration (C), diafiltration (D), and retentate collection by air purge (E) or buffer flush (F) process steps. Note: the color scheme and/or flow path shown in (A) will differ when prewashing is "off".

When the balance is connected to a local area network and remote access is enabled, it is possible to monitor TFF processes via a supported web browser.

5.5 Finishing a TFF Process

At the end of a TFF process, the user can choose what to do with the residual retentate in the cassette(s) and tubing.

It is common practice in TFF to perform a buffer flush. This can maximize molecule recovery and yield but dilute the retentate. An air purge is an alternative retentate collection method that can increase recovery rates while avoiding sample dilution.

To collect the residual retentate:

1. Press AIR PURGE or BUFFER FLUSH
2. Follow the on-screen instructions to adjust the flow path
3. Press OK [✓] to start retentate collection

For flow path diagrams, see 3.1 Hardware and Consumables, Figure 3.

Alternatively, when molecule yield is not important, the residual retentate may be left in place and discarded with the consumable waste.

To finish a process without collecting the residual retentate:

1. Press SKIP & FINISH

6. Process Reports

When each TFF process has finished, a process report is displayed on the balance (Figure 14). This includes some or all of the process parameters and results summarized in Table 6.

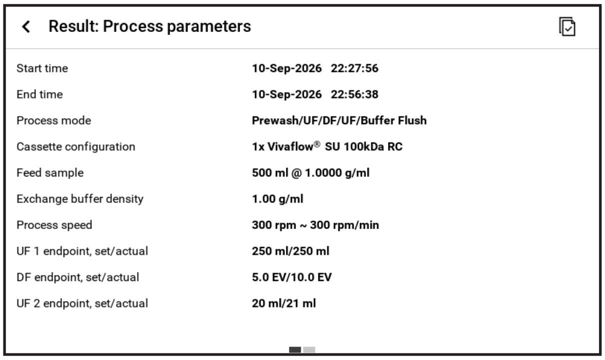

Figure 14: Example process report. Swipe to scroll through the configuration and results.

Process Report Content
Start and end date/time
Process mode (including prewashing and retentate collection settings)
Cassette configuration
Feed volume and density
Exchange buffer density
Process speed (per process step, configured and actual)
Average process rate (per UF/DF process step)
Endpoint (per UF/DF process step, configured and actual)

Table 6: Summary of process report content. Actual content varies based on the process configuration.

Process reports can be exported to various formats such as CSV or PDF, to support record keeping, SOP development, reproducibility and publication of experimental results. Reports can also be printed wirelessly to the lab printer (order number YDP30-NET, sold separately).

7. Software Calculations

While only permeate mass is measured by the system, accurately reaching the defined endpoint for each process step can be important. Therefore, as well as the feed volume and endpoint configuration, information about the cassette configuration and the solution densities are used by the software to calculate target permeate mass. In addition, the software continuously calculates the time remaining in each process step and uses this information to automatically reduce the pump speed as the defined endpoint approaches. Through this combination of user inputs and system features, the endpoint of each process step is reached with a high level of accuracy.

Calculations used by the software to determine target permeate masses are described in the following sections.

7.1 Permeate Mass for a Concentration Factor

When an ultrafiltration process has a concentration factor endpoint, the software uses the following calculation to determine the target permeate mass:

$$m_p = ((V_f - (V_f \div CF)) - V_h) \times \rho_f$$

Where m_p is the permeate mass, V_f is the feed volume, CF is the concentration factor, V_h is the cassette hold up volume, and ρ_f is the feed density.

7.2 Permeate Mass for a Retentate Volume

When an ultrafiltration process has a retentate volume endpoint, the software uses the following calculation to determine the target permeate mass:

$$m_p=((V_f-V_r)-V_h)\times\rho_f$$

Where m_p is the permeate mass, V_f is the feed volume, V_r is the retentate volume, V_h is the cassette hold up volume, and ρ_f is the feed density.

7.3 Permeate Mass for a Number of Exchange Volumes

When a diafiltration process has a number of exchange volumes as the endpoint, the software uses the following calculation to determine the target permeate mass:

$$m_p = (V_f \times \rho_f) + ((n_e-1)\times(V_f \times \rho_e)) - V_h$$

Where m_p is the permeate mass, V_f is the feed volume, ρ_f is the feed density, n_e is the number of exchange volumes, ρ_e is the exchange buffer density, and V_h is the cassette hold up volume.

7.4 Permeate Mass for an Exchange Efficiency

When a diafiltration process has an exchange efficiency endpoint, the software uses the following calculation to determine the required exchange buffer volume:

$$n_e = \frac{\ln(1 - E\%)}{T}$$

Where n_e is the number of exchange volumes, $E\%$ is the exchange efficiency, and T is the transmission coefficient.

The calculated number of exchange volumes is then used to determine the target permeate mass according to the calculation in **7.3 Permeate Mass for a Number of Exchange Volumes.**

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
3874 Research Park Drive
Ann Arbor, MI 48108
Phone +1 734 769 1600

For further information, visit
sartorius.com

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