EXOFASTER-500 QUICK GUIDE

Preparation and Confirmation of Consumables

- Chromatography column preparation and confirmation: ensure there are no large air bubbles or gel fractures.
- 1 mL pipette and tips, 1.5/2 mL microcentrifuge tubes, 0.22 μm syringe filters (optional).

Preparation of Reagents, Consumables, and Instruments

- Reagent preparation

	Reusable Kit (standard)	Non-Reusable Kit (standard)
Equilibration Buffer	(20X) 1 x 100mL: Dilute with ddH_2O to 1X.	(1X) 2 x 120mL
Elution Buffer	(8X) 2 x 120mL: Dilute with ddH_2O to 1X	(1X) 1 x 100mL
Blocking Buffer	Prepared by user: Mix 800mL ddH_2O with 200mL absolute ethanol	-
Wash Buffer	Prepared by user: Dissolve 1.6g NaOH in ddH_2O and bring volume to 1,000mL	-

*(*Storage Requirements: Chromatography columns and concentrated/reconstituted reagents can be stored at a temperature of 2-30°C. Reconstituted buffers are recommended to be stored long-term at 2–8 °C. Used columns should also be stored at 2–8 °C)*

- Connect elution buffer and equilibration buffer tubing (Optional: connect blocking and wash buffer tubing if using the reusable kit)
- Confirm waste liquid tubing connection
- Plug in the power supply unit
- Press 'Initialize Instrument' at least two times to ensure liquid is dispensing from the corresponding needles. Needles (from left to right): Blocking Buffer, Wash Buffer, Equilibration Buffer, Elution Buffer.

Instrument Operation

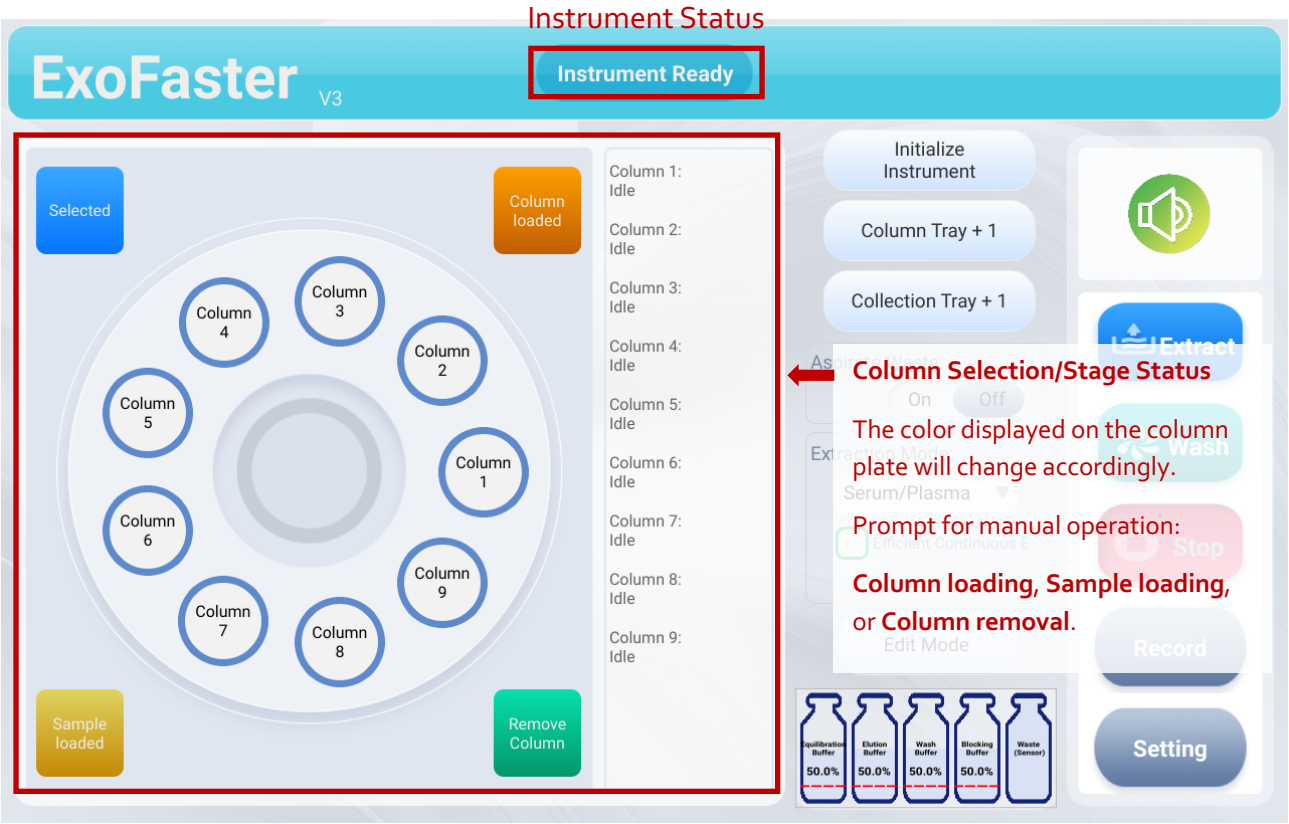


Figure 1

Instrument Initialization:
Perform the instrument initialization procedure.

Rotate Column Tray +1:
Rotate the column tray clockwise by one position.

Rotate Collection Tray +1:
Rotate the collection tray clockwise by one position.

Waste Extraction Switch:
Set the waste extraction function to **ON** or **OFF**.

Extraction Mode:
Allow the user to select the desired extraction mode.

Edit Mode:
Enter the sub interface, edit extraction modes.

Monitoring:
Monitoring the buffer volume.

Sound
Turn on/off prompt sound.

Extraction:
Begin the extraction procedure.

Wash:
Begin the post isolation column wash procedure.

Stop:
Stop the current procedure.

Record:
Search for experimental records and export data.

Maintenance:
Enter the maintenance interface.

Figure 2

EV Extraction

Available Modes:

	Default Sample Volume*	Default Fraction Collected
Serum/plasma EV Isolation	1mL	Fraction 7-8
Cell Culture Supernatant EV Isolation	1/1.5mL	Fraction 6-8
Custom EV Isolation	1mL	Fraction 7-8
1vN EV Isolation	N/A (Acceptable Range: 0.5-2mL)	User-selectable fraction 1–18

**For serum/plasma samples, the recommended loading volume is 200μL - 1.0 mL. For cell culture supernatant and other sample types, the default loading volume is 1 or 1.5 mL. The current program is configured based on the default loading volume.*

Efficient Continuous Extraction *can be selected in all modes except 1vN. This function allows the same column to be loaded multiple times, up to 5 times. A simple washing step is completed during each loading cycle. The loading volume range follows the rules for each sample type specified above.*

If you need to change the loading volume, please inform us, and our Technical Support team will provide the required parameter values. You can then create a new profile using these updated settings. Different loading volumes may result in different fraction collection volumes, so parameter optimization is necessary.

Procedures:

- Switch on power to enter the main program interface automatically.
- Click on any column position button to enter the column setting page (See Figure 3).

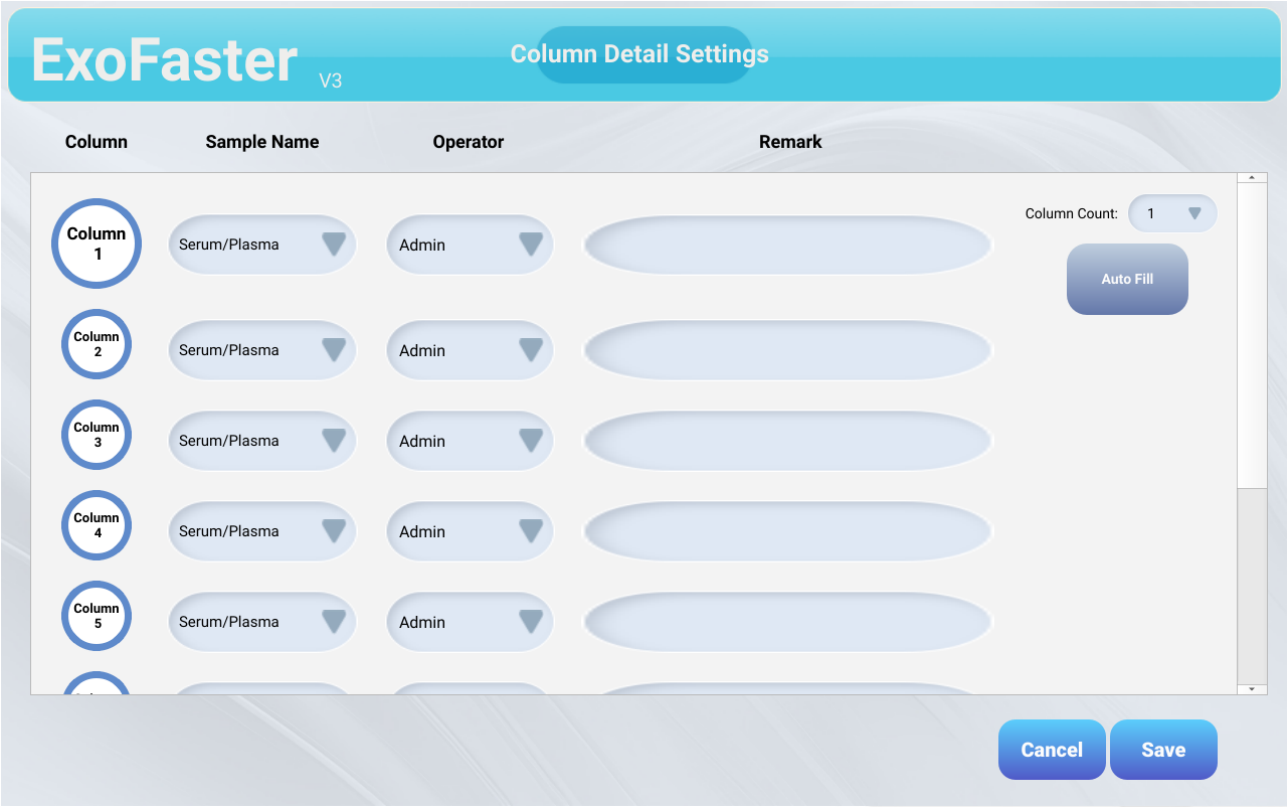


Figure 3

- Set the position and other information where the chromatography column will be placed. Select 'Auto Fill' and specify 'Column Count' to match the number of columns to be run. Click 'Save' to save the information and return to the main program interface.
- Place 1.5/2 mL microcentrifuge tubes in corresponding collection tray.
- Select the extraction mode, then click **Extract**. Click **OK** to confirm and start the extraction (See Figure 4).

Figure 4

- Follow UI prompts:

1. Once the run begins, the system prompts for column insertion and indicates the position to load the column (See Figure 5).

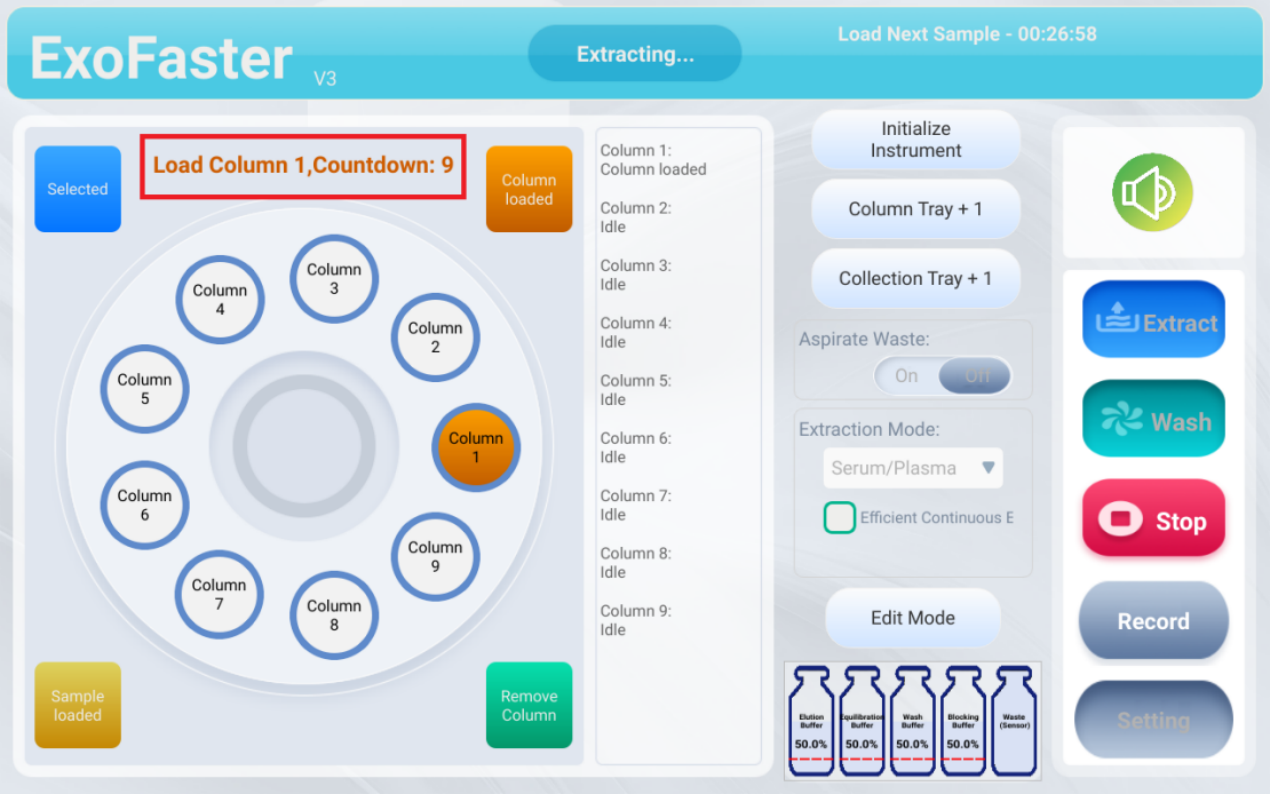


Figure 5

2. Remove the top/bottom caps fom the column before loading (See Figure 6). Insert the columns sequentially, one at a time.

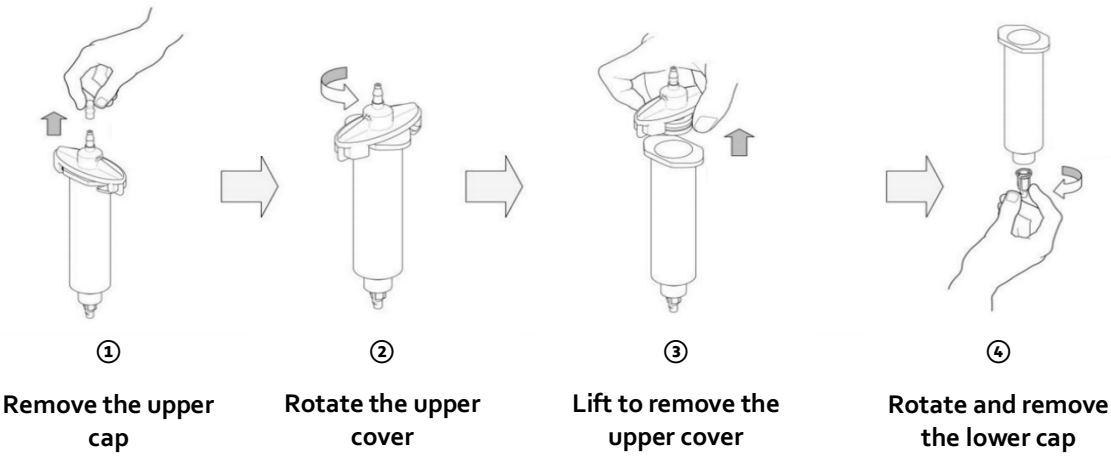


Figure 6

3. After ~26 min: the system prompt for addition of sample. Load each sample sequentially, one at a time (See Figure 7).

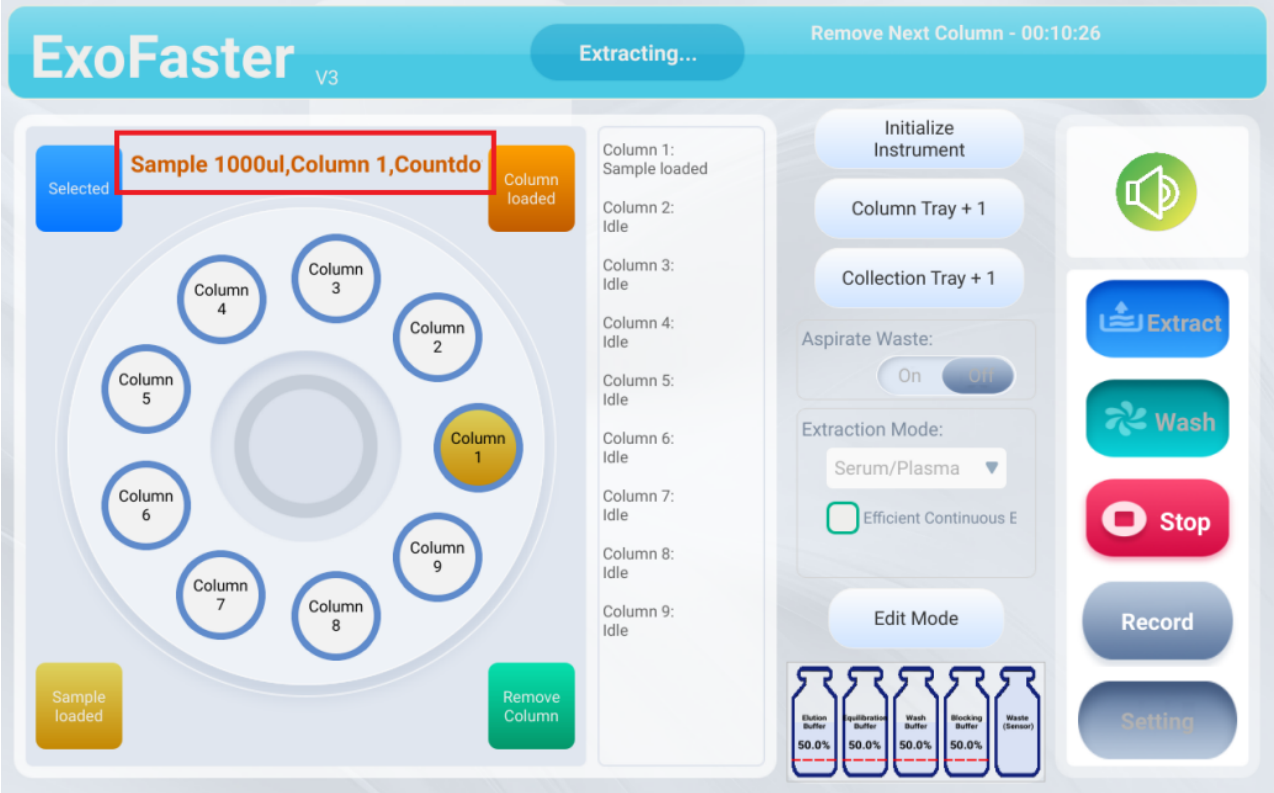


Figure 7

4. After ~10 min: collection is complete and the system prompts for column removal (See Figure 8).

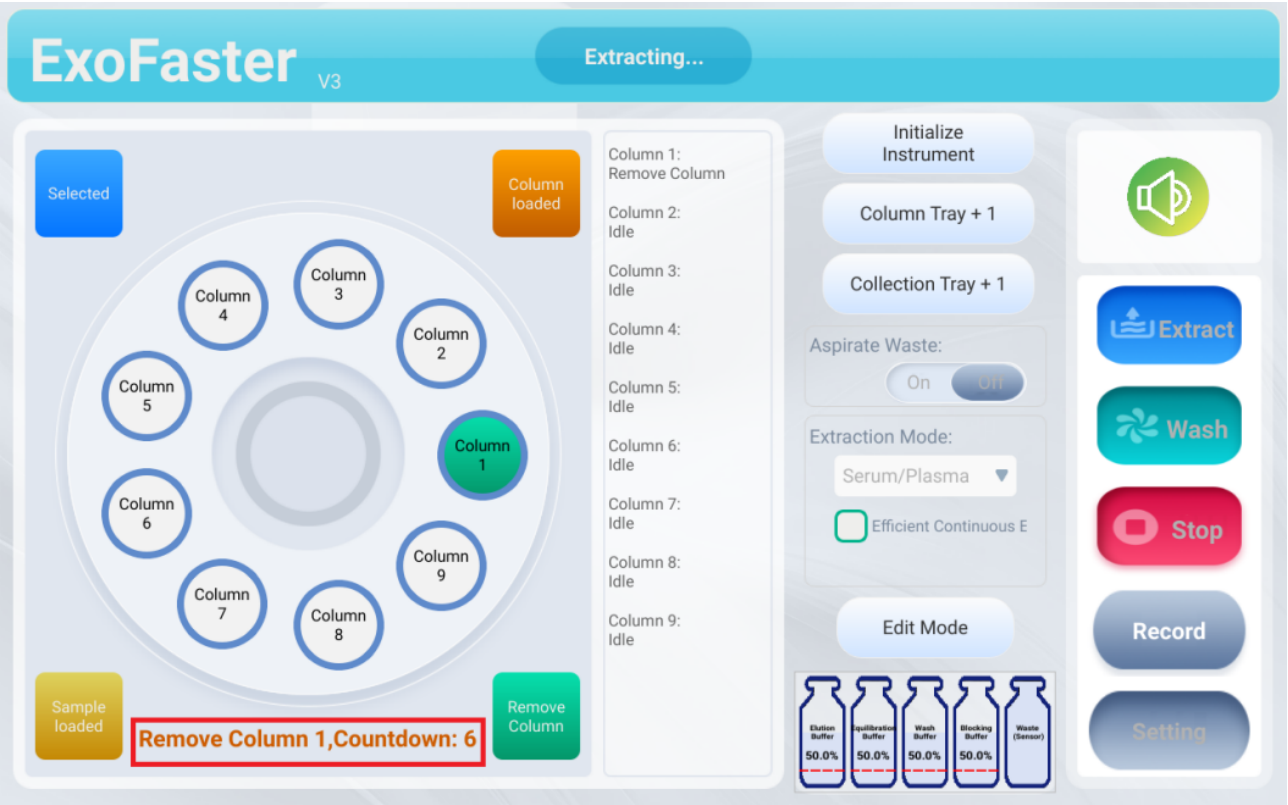


Figure 8

*When configuring the extraction mode, if 'Efficient Continuous Extraction' is selected, the 'Times' parameter is set, and the number of selected column positions is 2 or more, the system will prompt you to replace the microcentrifuge tube(s) after each collection cycle (See Figure 9). After replacing the tube(s), repeat the sample addition and collection steps until the total number of sample additions reaches the set Times value.

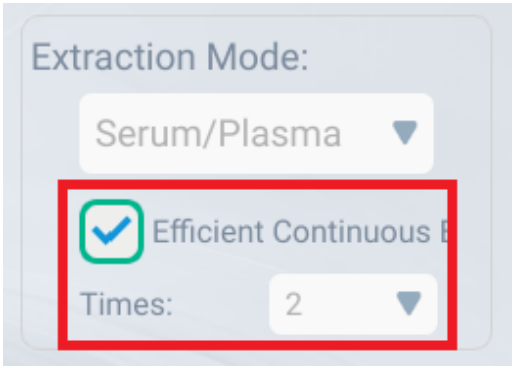
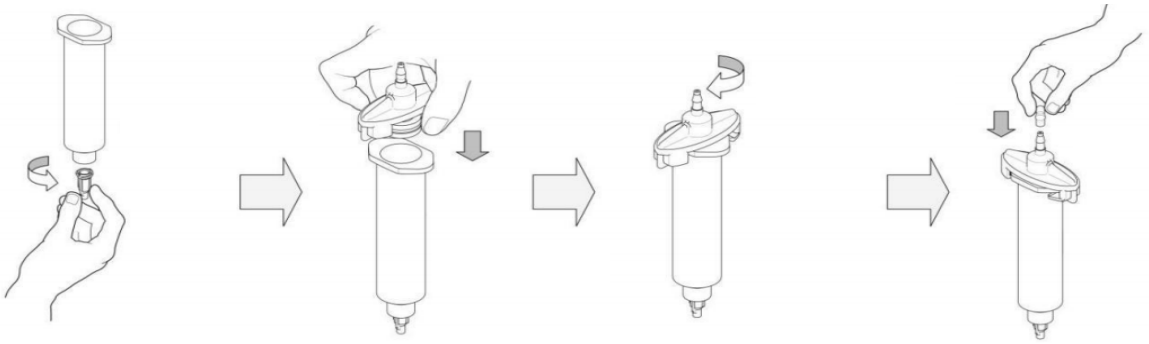


Figure 9

5. Replace the top and bottom caps on the column after unloading (See Figure 10).



① **Rotate to close the lower cap**

② **Press down to secure the upper cover**

③ **Rotate to tighten the upper cover**

④ **Replace the upper cap**

Figure 10

6. Retrieve the collection tubes containing EVs from the collection tray.

Record Checking

1. In the Record section, search for past run records by applying the required filters, then click 'Query' to search (See Figure 11).
2. To export the results to a USB drive, click 'Export'.

Figure 11

Post-Run Column Wash

Procedures (**Reusable columns**):

- Select the column position where the column will be placed.
- Click **Wash**. Click **OK** to confirm and start column wash (See Figure 12).

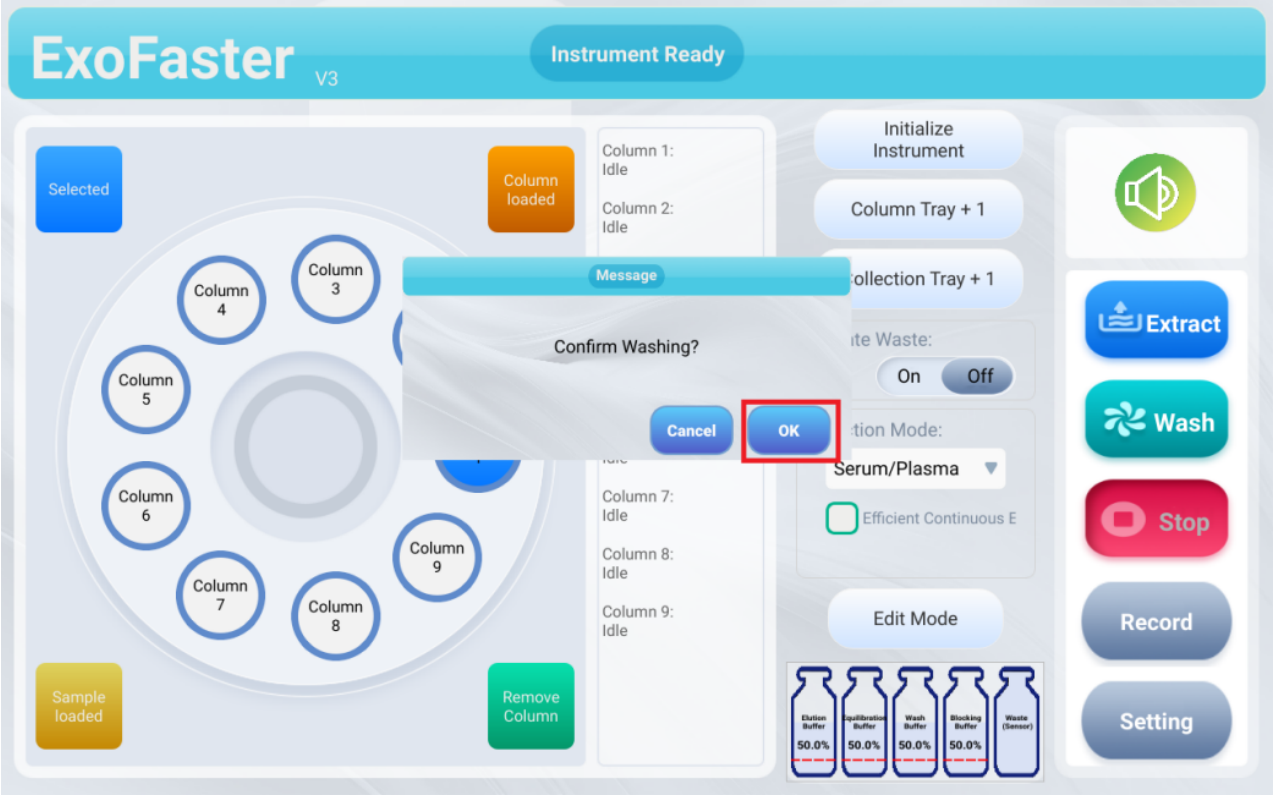


Figure 12

- Follow UI prompts:

1. Once the run begins, the system prompts for column insertion and indicates the position to load the column.
2. Remove the top/bottom caps fom the column before loading (See Figure 6). Insert the columns sequentially, one at a time.
3. After ~24 min: washing is complete and the system prompts for column removal.
4. Replace the top and bottom caps on the column after unloading (See Figure 10).

Procedures (**Non-reusable columns**):

- Non-reusable columns are single-use and not intended for reuse, no post-run column washing step is required. After the run, dispose of the columns in accordance with local regulations and institutional guidelines appropriate to the sample type processed.

Long-Term Idle Flush Procedure

If the instrument will be unused for more than one week, empty remaining reagents and flush the fluid lines to prevent clogging.

Materials:
- Clean beaker
- Connecting tubes (available in accessory kit)
- Purified water

Steps:

1. Fill the beaker with sufficient purified water.
2. Place the ends of all connection tubes into the same beaker of purified water (ensure the tube ens are submerged), then connect the other ends to the corresponding buffer ports on the right-hand side of the instrument.
3. Click “Initialize Instrument” 3 times until all 4 dispensing needles finish dispensing.
4. Remove all tube ends from the beaker of purified water.
5. Cick “Initialize Instrument” 2 times with the tube ends not submerged in purified water to drain and dry the tubes.
6. Disconnect the 4 tubes from the instrument, wipe dry, and store each tube in a sealed bag for future use.

Notes

- When running the selected extraction mode, please load the sample according to the required loading volume. Do not use non-default sample volumes for the selected extraction mode. If the sample volume is insufficient, top up with 1× PBS. If the sample volume exceeds the required loading volume, please concentrate the sample or take the appropriate volume for loading.
- Whether loading the sample or adding different reagents, make sure that there is no residual liquid on the sieve plate of the column (at this point, no liquid should be coming out of the bottom of the column) before proceeding.
- Avoid allowing the columns to dry out after use, as this may result in poor performance.
- For non-standard samples, run 1vN workflow and retrieve up to 18 fractions (0.5 mL each) to determine suitable fractions by EV/protein concentration.
- For optimization of non-standard samples, contact technical support.