

QUICK START GUIDE

AUTOMATIC FRACTION COLLECTOR V2 (AFC V2)



This quick start guide only provides general operating instructions. For more detailed information, download the AFC V2 User Manual at support.izon.com

INTENDED USE

The AFC is used to automate the collection of extracellular vesicle (EV)-containing volumes from qEV columns. The instrument is intended for use by professional personnel only.

COMPONENTS OF THE AFC SYSTEM

1. AFC instrument with attached Silicone Waste Tube
2. Nozzle Set (x8)
3. qEV Column Mount (x5)
4. Carousel Cover
5. Reversible Carousel
6. Carousel Plate
7. qEVoriginal Reservoir
8. 5 mL Collection Tube (x15)
9. 2 mL Collection Tube (x30)
10. 12 Volt Power Supply
11. Power Lead
12. USB 2.0 Type A to USB 2.0 Micro Cable (male to male)
13. 10 g Calibration Weight



The carousel is reversible and can be used in two orientations:

- ▶ One side has seven large holes and uses 5 mL collection tubes.
- ▶ One side has thirteen small holes and uses either 1.5 mL or 2.0 mL collection tubes. Cannot be used with the qEV10.
- ▶ The qEV100 is not compatible with the AFC.

To complete a run on the AFC you will also need:

A qEV column, pipettes and tips, buffer (freshly prepared, 0.22 μm filtered, and degassed), freshly prepared 0.5 M NaOH solution and 70% ethanol solution for column cleaning, and a computer with Windows 10 for updating the AFC operating firmware as required.

INSTALLING THE NOZZLE SET

After the instrument has been assembled on a level surface, install the nozzle set. To learn how to install the nozzle set, visit support.izon.com/how-to-install-the-afc-nozzle-set for a demonstration.

ALIGNING THE CAROUSEL

Next, align the carousel with the fluid nozzle. To align the carousel press **Settings** on the left-hand side of the home screen, then choose **Carousel Calibration** and follow the on-screen prompts.

CALIBRATING THE SCALE

From the home screen, press **Settings** > **Scale Calibration**, and follow the instructions.

AFC OPERATION

MOUNTING THE COLUMN TO THE AFC TOWER



The AFC will only work with qEV columns.

1. Turn on the instrument using the power switch at the rear.
2. Check the waste tube to ensure it empties into a sink or waste vessel.
3. Before a column has been inserted, the tower lighting will be white, and the home screen will display "No Column".



A warning indicator will be displayed when the recommended number of uses (5) has been exceeded. After 5 uses, the warning indicator will appear and a countdown from 5 to 0 uses will commence. The AFC will display "invalid column" when it has been used a total of 10 times.

4. Fit the appropriate column mount onto the AFC tower.
5. If using a qEVsingle, qEVoriginal, or qEV1, remove the top cap before placing the column in the column mount. If using a qEV2 or qEV10, fit the reservoir to the column, remove the lower cap and ensure the fluid is flowing properly before placing the column in the column mount.
6. Ensure the lower cap has been removed, insert the column into the column mount and carefully dock the column. Ensure that the Izon logo on the column is visible and the AFC can read the RFID tag.
7. The qEV column type will be displayed on the screen. If not, rotate column until displayed.
8. Ensure connection between the column and the tube adapter is secure to avoid leaks.



To minimise buffer being spilled while connecting the column to the tube adapter, make the connection quickly and wipe any drops that land on the AFC.

ADJUSTING THE COLLECTION SCHEDULE

Once the appropriate column has been selected and mounted to the AFC tower, the collection schedule must be set.

1. From the home screen, you can adjust the count, volume, and buffer volume for collection. Press **-/+** and follow the on-screen prompts to save your parameters.

SETTING UP THE CAROUSEL

1. Load the collection tubes onto the carousel with the lids facing inwards. Ensure you have loaded enough tubes for your collection protocol.
2. Gently place the carousel onto the carousel plate. Ensure that the carousel alignment hole engages with the raised carousel alignment pin on the carousel plate.



Remember to label your tubes before placing them into the AFC carousel.

FLUSHING THE COLUMN

1. The next screen will ask you to mount the column reservoir. Note: If using a qEVsingle, a column reservoir is not needed.
2. You will now be asked if you wish to flush the column. If this is your first use of the column for the day, we recommend flushing of all columns with fresh, filtered PBS buffer. Press **Yes** to continue, load the recommended volume of buffer and press **OK** to continue. If you select **No** the next screen will ask you to load 1 mL of buffer onto the column to prime the nozzle. Press **OK** to continue.

SAMPLE LOADING AND INITIATING COLLECTION

1. The AFC will ask you to have sufficient buffer available to run the column and to load the sample.
2. Ensure that there is no residual buffer left on the top of the column, remove any excess with a pipette. Now add your sample.



It is important to only load the sample at this point. Adding buffer at the same time or before the sample has fully entered the loading frit will result in incorrect sample loading.

3. Press **Start** to continue. The column will start running and the carousel will move to the buffer collection position. The lighting will change to **red** to indicate that the collection has started.
4. When the sample has fully entered the loading frit, top up the column/reservoir with the correct amount of buffer to resume collection of the buffer volume.

5. When the buffer volume has been collected, the carousel will move to position 1, collect the specified volume and then progressively move to collect each successive individual fraction. If more collection tubes are required than a fully loaded carousel, the user will be instructed when to remove the cover and carousel, replace the tube positions with fresh tubes and continue the run.

COMPLETING A RUN

After a run has been completed, it is important to clean the column.

1. When the final fraction has been collected, the lighting will turn **green**. The carousel cover and the collected fractions can now be safely removed.
2. We recommend cleaning the column after every use. Select **Clean** to be guided through the cleaning process. When the cleaning process is complete, the lighting will turn **green**; press **OK** to return to the home screen. To flush the column without cleaning, select **Flush**.



Remember to empty and clean out the carousel central well between consecutive runs.

AFC SHUT DOWN



The nozzle should be flushed thoroughly with 70% ethanol solution after the last run of the day to prevent the growth of bacteria within the tubing.

1. Before turning off the AFC, rinse the AFC nozzle using DI water with the valve open. This will prevent salt crystallising in the tip of the nozzle while it is not in use.
2. Switch off the AFC at the rear of the instrument.

For more detailed instructions, refer to the AFC V2 User Manual available at support.izon.com/automatic-fraction-collector-v2-user-manual

If your AFC needs replacement parts or repair please contact your regional Izon representative or open a support ticket at support.izon.com