

AUTOMATIC FRACTION COLLECTOR



USER MANUAL



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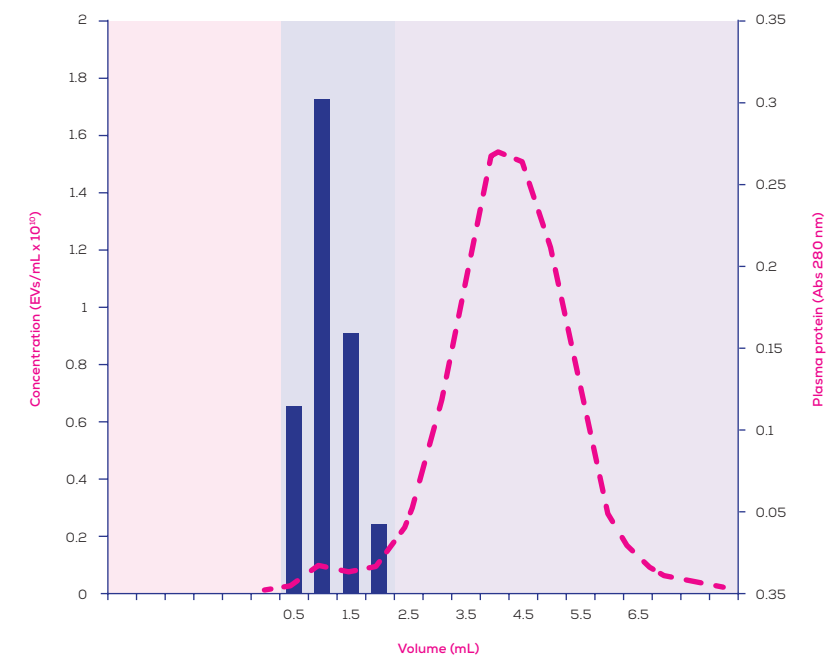
INTRODUCTION

Isolation of extracellular vesicles (EVs) using the combination of qEV and Automatic Fraction Collector (AFC) technologies enhances the precision of collecting the EV containing fractions, eliminating the tedium and the inherent inaccuracy of manually collecting EV fractions. The AFC offers users the capacity to scale up EV isolation through streamlining the equilibration, vesicle elution and washout and regeneration of the columns. Multiplexing AFCs means that labs can process large numbers of EV samples.



AFC BASICS AND PRINCIPLES

The AFC manages the elution of EVs by discriminating the void zone from the EV zone. The void volume is the zone designated in red, consisting of the volume of fluid preceding the EV elution. The void volume does not contain any EVs and is not retained within any Eppendorf tubes. Instead the void volume is collected into the central well of the AFC carousel. Once the void volume has been collected, the eluate containing EVs is collected into Eppendorf tubes. The EV collection zone in Figure 1 is indicated in green. The protein zone after the collection of EVs may also be collected if required.



SET-UP

Unpack and set-up AFC

Unpack the AFC and install on the laboratory bench. The instrument should not be set up close to any equipment that causes vibrations, such as centrifuges, culture & plate shakers, vortex mixers, sonication devices etc. The AFC's load cell measures the weight of the fractions and any vibrations might affect its proper functioning. The waste tube from the AFC wash can exit from the rear or the side of the instrument into a sink or a waste bottle. The 12 volt power supply plugs into the rear of the instrument.

How to use the AFC

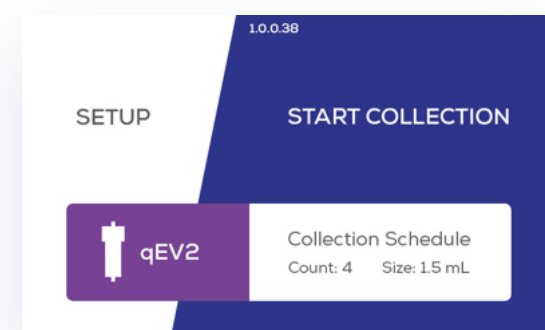
Plug in the power supply and turn on the instrument with the switch at the rear. The screen will light up, briefly showing the IZON logo and will then ask you to ensure the blue manual valve at the front of the AFC is open. The valve is open when the lever is vertical.



Press **OK** to continue.

Column Setup & Collection Schedule

The next screen allows you to select the column type and fractions.



If the column displayed is not the column type you wish to use, press the left-hand column selection zone until the correct column type is displayed. The display will cycle through the different column types. For each column type there are two colour types, purple is for the qEV70 column series and blue for the qEV35 column series. The column colour selected in the screen should match the colour branding on the column.

The right-hand window of the same screen enables you to select the fraction number and fraction size you wish to use. For each column type (except the qEVsingles) there are a number of fraction size options available. Press the **COLLECTION SCHEDULE** button and select the fraction options you require. Pressing +/- enables you to select your collection options. Press the **X** to exit out of this screen.

Inserting column

The AFC comes with individual column mounts to match the different qEV options. For each column type there are detailed sections below referring to the column type of interest. Install the correct column holder onto the tower of the AFC.

To insert the required column into its holder on the AFC tower, remove the lower luer cap of the column and insert the column from above into the holder and dock the column with the female luer fitting on top of the blue manual valve. Installing a column into the holder and docking with the luer connector on the instrument may lead to a few drops of buffer spilling onto the instrument. Minimise this by making the connection quickly and wipe up any drops. Make sure the connection between the column and the valve is secure to avoid any leakage.

The AFC measures fluid volume by accurately determining volumes by weight. When the designated threshold weight is reached, the fluid flow is stopped automatically to prevent dripping of fluid during repositioning of the carousel.

Collection process

Press **START COLLECTION** to progress to the next screen. This window simply confirms the selected fraction counts and fraction sizes are correct. Press **YES** to continue, press **NO** if you want to change the collection options.

Setup carousel

The next window will instruct you to prepare the carousel for collection. The carousel can be used in two orientations. One side has a series of large holes and uses 5 mL Eppendorf tubes and is only used for the qEV10 column. The reverse side uses 1.5 or 2 mL tubes. Only the qEV10 column uses the carousel with the 5mL tube options. In this orientation the lids of the 5 mL tubes must not make contact with the carousel cover (see below qEV10 section). For qEV2 columns, 2 mL tubes must be used when the standard 2 mL fraction size is selected.

Always load the required tubes into the carousel with their lids facing inwards and then gently place the carousel onto the AFC.

The carousel has a small locating hole on its rim, this must engage with the raised pin on the AFC weigh plate, shown below.



Press **OK** to continue and go to the appropriate sections below for the different column type you are wanting to run.

qEVORIGINAL OPERATION ON AFC

The qEVoriginal uses the carousel side with the small holes containing 1.5 or 2 mL Eppendorf tubes. The screen now asks you to mount the buffer reservoir onto the column. A qEVoriginal reservoir is supplied with the AFC. Image of the qEVoriginal with its reservoir is shown below.



Press **OK** to continue.

The next window will ask you whether you would like to flush the column. We recommend flushing of all columns with fresh filtered PBS buffer. If a column has been used and flushed and another flush is not required press **NO**. To flush press **YES**.

The flush volume for the qEV original is 15 mL. When the AFC is in flushing mode, the flush buffer flows out of the column and through the noncircular hole in the carousel.

At the base of the AFC there is a clear silicon tube. This must be draining into a sink or a suitable waste reservoir close to the AFC.

Press **OK** to continue and the waste pump will commence operating. Press **OK** when sufficient flushing buffer has flowed through the column.

The next screen will ask you to place the carousel cover onto the AFC. The cover protects the carousel from being inadvertently bumped during operation. Tube lids must not make contact with the carousel cover.



Press **OK** to continue

The next screen asks you to remove any residue buffer from the top of the column. Press **OK** to continue.

The next screen asks that you have sufficient buffer to run the column and to load the sample. The optimal sample loading volume for qEVoriginal is 0.5 mL. The amount of buffer required to run the qEVoriginal column is 15 mL. Press **OK** to continue.

The column will start running, the AFC carousel will move to the void collection position and the fluid will collect into the central large well of the carousel. The lighting will change to red indicating that the process has commenced and the user should not bump or interfere with the carousel. When the sample has fully entered the upper frit of the column, top up the reservoir with buffer and press **OK** to continue.

When the void volume has been collected the carousel will move to tube 1, collecting the specified volume and then progressively move to collect each individual successive fraction. If more fraction tubes are required

than the fully loaded carousel, the user will be instructed when to remove the cover and carousel, replace the tube positions with fresh tubes, replace the carousel and cover and continue the run.

When the final fraction has been collected, the display lighting turns green and the cover and carousel can be removed and fractions can be recovered.

The user now selects **CONTINUE** or **FINISH**. If the user wants to continue with another run a flush cycle must occur to remove contaminating proteins and biological material from the column.

It is important to empty and clean out the carousel between consecutive runs, since there is a limit to the total weight at which the load cell can measure accurately.

If the user selects **FINISH**, flushing of the column is recommended to maintain the column in good uncontaminated condition and the column should be stored at 4°C. Note, if the AFC is turned off with a column still attached, the blue valve must be closed to prevent fluid flow.

Table 1: qEVoriginal specifications

Column name	qEVoriginal	
Column series	qEVoriginal / 70nm	qEVoriginal / 35nm
Optimal separation size	70-1000 nm	35 -350 nm
Nominal flow-rate (mL/min at 20°C)	1.0	0.6
Sample load volume (mL)	Up to 0.5	
Column volume (mL)	10	
Optimal fraction size (mL)	0.5	
Void volume (mL)	2.85	
Flush volume (mL)	15	

qEVSINGLE OPERATION ON AFC

The qEVsingle is a single use disposable column for small volume EV purification and is ideal for applications where the downstream EV analysis involves nucleic acid amplification, e.g. PCR or RT-PCR where cross-contamination from sample to sample is to be avoided.

The qEVsingle uses the carousel side with the small holes containing 1.5 or 2 mL Eppendorf tubes. The qEVsingle does not come with its own reservoir as the column body has ample empty space above the resin to accommodate sufficient fluid for elution of the EV fraction.

Press **OK** to continue.

The next window will ask you whether you would like to flush the column. We strongly recommend flushing of all columns with fresh filtered PBS buffer.

The flush volume for the qEVsingle is 4 mL. When the AFC is in flushing mode, the flush buffer flows out of the column and through the noncircular hole in the carousel. At the base of the AFC there is a clear silicon tube. This must be draining into a sink or a suitable waste reservoir close to the AFC. Press **OK** to continue and the waste pump will commence operating. Press **OK** when sufficient flushing buffer has flowed through the column.

The next screen will prompt the user to place the carousel cover onto the AFC. The cover protects the carousel from being inadvertently bumped during operation. Press **OK** to continue

The next screen asks you to remove any residue buffer from the top of the column. Press **OK** to continue.

The next screen asks that you will have sufficient buffer to run the column and to load the sample. The optimal sample loading volume for qEVsingle is up to 0.15 mL. The amount of buffer required to run the qEVsingle column is 4 mL. Press **OK** to continue.

The column will start running. The AFC carousel will move to the void collection position and the fluid will collect into the central large well of the carousel. The lighting will change to red indicating that the process has commenced and the user should not bump or interfere with the carousel. When the sample has fully entered the upper frit of the column top up the column with buffer and press **OK** to continue.

When the void volume has been collected the carousel will move to tube 1, collecting the specified volume and progressively move to collect each individual fraction. If more fraction tubes are required than the 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.

When the final fraction has been collected, the display lighting turns green and the cover and carousel can be removed and fractions can be recovered.

You can select **CONTINUE** or **FINISH**. If you want to continue with another qEVsingle run, a new column should be placed on the AFC. It is important to empty and clean out the carousel central well between consecutive runs.

If you select **FINISH** and the AFC is turned off, the blue valve should be closed to prevent fluid flow.

Table 2: qEVsingle specifications.

Column name	qEVsingle	
Column series	qEVsingle / 70nm	qEVsingle / 35nm
Optimal separation size	70-1000 nm	35 -350 nm
Nominal flow-rate (mL/min at 20°C)	0.25	0.17
Sample load volume (mL)	Up to 0.15	
Column volume (mL)	3.5	
Optimal fraction size (mL)	0.2	
Void volume (mL)	1.25	
Flush volume (mL)	3.5-4	

qEV2 OPERATION ON AFC

The qEV2 is optimized for 2 mL sample loading. The qEV2 uses the carousel side with the small holes containing 2 mL Eppendorf tubes. If the selected fraction size is 1.5 or less 1.5 mL Eppendorf tubes may be used. The AFC screen now asks you to mount the buffer reservoir onto the column. The qEV2 reservoir is supplied with the columns.

Press **OK** to continue.

The next window will ask you whether you would like to flush the column. We strongly recommend flushing of all columns with fresh filtered PBS buffer. If a column has been used and flushed and another flush is not required press **NO**. To flush press **YES**.

The flush volume for the qEV2 is 60 mL. When the AFC is in flushing mode, the flush buffer flows out of the column and through the noncircular hole in the carousel. At the base of the AFC there is a clear silicon tube. This must be draining into a sink or a suitable waste reservoir close to the AFC. Press **OK** to continue and the waste pump will commence operating. Press **OK** when sufficient flushing buffer has flowed through the column.

The next screen will the user to place the carousel cover onto the AFC. The cover protects the carousel from being inadvertently bumped during operation. Press **OK** to continue.

The next screen asks you to remove any residue buffer from the top of the reservoir. Press **OK** to continue.

The next screen asks that you will have sufficient buffer to run the column and to load the sample. The optimal sample loading volume for qEV2 is 2 mL. The amount of buffer required to run the qEV2 column is 60 mL. Press **OK** to continue.

The column will start running, The AFC carousel will move to the void collection position and the fluid will collect into the central large well of the carousel. The lighting will change to red indicating that the process has commenced and the user should not bump or interfere with the carousel. When the sample has fully entered the upper frit of the column top up the reservoir with buffer and press **OK** to continue.

When the void volume has been collected the carousel will move to tube 1, collecting the specified volume and progressively move to collect each individual fraction. If more fraction tubes are required than the 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.

When the final fraction has been collected, the display lighting turns green and the cover and carousel can be removed and fractions can be recovered.

You can now select **CONTINUE** or **FINISH**. If you want to continue with another run a flush cycle must occur to remove contaminating proteins and biological material from the column. It is important to empty and clean out the carousel between consecutive runs, since there is a limit to the total weight at which the load cell can measure accurately.

If the user selects **FINISH**, flushing of the column is recommended to maintain the column in good uncontaminated condition and the column should be stored at 4°C. Note, if the AFC is turned off with a column still attached, the blue valve must be closed to prevent fluid flow.

Table 3: qEV2 specifications

Column name	qEV2	
Column series	qEV2 / 70nm	qEV2 / 35nm
Optimal separation size	70-1000 nm	35 -350 nm
Nominal flow-rate (mL/min at 20°C)	4.5	2.5
Sample load volume (mL)	Up to 2	
Column volume (mL)	47	
Optimal fraction size (mL)	2	
Void volume (mL)	14.25	
Flush volume (mL)	60	

qEV10 OPERATION ON AFC

The qEV10 is optimized for 10 mL sample loading. The qEV10 uses the carousel side with the large holes containing 5 mL Eppendorf tubes. **Note, it is very important that the lids of the 5 mL tubes are not making contact with the carousel cover.** Bend the tubes lids so they are sitting flat on the carousel and check that there is no contact when placing the carousel cover on.

The screen now asks you to mount the buffer reservoir onto the column. The qEV10 reservoir is supplied with the columns.

Press **OK** to continue.

The next window will ask you whether you would like to flush the column. We strongly recommend flushing of all columns with fresh filtered PBS buffer. If a column has been used and flushed and another flush is not required press **NO**. To flush press **YES**.

The flush volume for the qEV10 is 120 mL. When the AFC is in flushing mode, the flush buffer flows out of the column and through the noncircular hole in the carousel. At the base of the AFC there is a clear silicon tube. This must be draining into a sink or a suitable waste reservoir close to the AFC. Press **OK** to continue and the waste pump will commence operating. Press **OK** when sufficient flushing buffer has flowed through the column.

The next screen will the user to place the carousel cover onto the AFC. The cover protects the carousel from being inadvertently bumped during operation. Press **OK** to continue.

The next screen asks you to remove any residue buffer from the reservoir. Press **OK** to continue.

The next screen asks that you will have sufficient buffer to run the column and to load the sample. The optimal sample loading volume for qEV10 is 10 mL. The amount of buffer required to run the qEV10 column is 120 mL. Press **OK** to continue.

The column will start running, The AFC carousel will move to the void collection position and the fluid will collect into the central large well of the carousel. The lighting will change to red indicating that the process has commenced and the user should not bump or interfere with the carousel. When the sample has fully entered the frit of the column reservoir, top up the reservoir with buffer and press **OK** to continue.

When the void volume has been collected the carousel will move to tube 1, after collecting the specified volume it will progressively move to collect each individual fraction. If more fraction tubes are required than the 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.

When the final fraction has been collected, the display lighting turns green and the cover and carousel can be removed and fractions can be recovered.

You can now select **CONTINUE** or **FINISH**. If you want to continue with another run a flush cycle must occur to remove contaminating proteins and biological material from the column. It is important to empty and clean out the carousel between consecutive runs, since there is a limit to the total weight at which the load cell can measure accurately.

If the user selects **FINISH**, flushing of the column is recommended to maintain the column in good uncontaminated condition and the column should be stored at 4°C. Note, if the AFC is turned off with a column still attached, the blue valve must be closed to prevent fluid flow.

Table 4: qEV10 specifications

Column name	qEV10	
Column series	qEV10 / 70nm	qEV10 / 35nm
Optimal separation size	70-1000 nm	35 -350 nm
Nominal flow-rate (mL/min at 20°C)	5.5	3.5
Sample load volume (mL)	Up to 10	
Column volume (mL)	74	
Optimal fraction size (mL)	5	
Void volume (mL)	20.25	
Flush volume (mL)	120	

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