SOP: Purification of AAV-GFP Viral Particles from HEK293F AAV GFP Cell Lysate by Affinity Chromatography on the ÄKTA Pure Chromatography System

Approvals

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1. **Purpose**
   1.1. This procedure describes the isolation of AAV GFP viral particle from HEK293F AAV GFP cell lysate affinity chromatography with the ÄKTA pure Chromatography System, controlled by Unicorn 6.3 software.
2. **Scope and Applicability**
   2.1. Applies to purification of AAV GFP viral particle from prepared HEK293F AAV GFP cell lysate, which has been filtered using 0.45um filter. The method employs a 1 ml HiTrap AVB Sepharose HP column installed on the ÄKTA pure Chromatography System and controlled by Unicorn 6.3 software.
3. **Summary of Method**
   3.1. Preparation of buffer(s)
   3.2. Priming the pump rinsing system
   3.3. Priming Inlets and purging pump heads
   3.4. Installing/changing of chromatography column (if required)
   3.5. Assembly and installation of a 10 ml Superloop sample chamber.
   3.6. Calibration of the pH electrode/detector
   3.7. Equilibration of system and column
   3.8. Fraction collector setup
   3.9. Application of sample to affinity column
   3.10. Washing and elution of column
   3.11. Regeneration of system in preparation for subsequent run
   3.12. Procedures for cleaning and short or long-term storage of the system
4. **References**
   4.1. Unicorn 6.3 Users Guide (electronic)
   4.2. AKTA pure 25 Users Guide (https://cdn.cytivalifesciences.com/api/public/content/digi-16308-original)
   4.3. https://www.cytivalifesciences.com/en/us/solutions/cell-therapy/knowledge-center/resources/Enhanced-AAV-downstream-processing
   4.4. Equipment SOP: Operation of ÄKTA pure Chromatography System, Document number: DP 5 revision number: 3 Effective Date 26 JUL21
   4.5. SuperLoop 10ml, User instructions https://cdn.cytivalifesciences.com/api/public/content/digi-12806-pdf
5. **Definitions**
   5.1. N/A

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6. Precautions

6.1. Routine care should be exercised in handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick, etc.

6.2. User should read and be familiar with general good practice as outlined in the AKTA pure Cue Cards located near the instrument.

6.3. Avoid damaging the threads through the use of excessive force when connecting plastic fasteners.

6.4. It is imperative that pumps never be allowed to run dry. Care must be taken to avoid air in the fluid path, which could damage the pumps or give spurious and uninterpretable readout from the UV and/or conductivity detectors. In order to prevent this prime the pump rinsing system, prime inlets and purge pump heads before each run by referring to the steps 9.5 and 9.6 of the SOP: Operation of ÄKTA pure Chromatography System.

6.5. Gloves and protective eyewear should be worn when handling samples and reagents (buffers), however it is preferable that the user remove gloves prior to entering commands via the computer keyboard or mouse.

6.6. Buffers must be degassed and filtered prior to use with the AKTA pure instrument. Samples should be, at a minimum, centrifuged at 10000x*g* for 5 min before injection/introduction into the fluid path.

6.7. Equipment calibration check: The AKTA pure system calibration is automatic; baseline for measurements of A280 and conductivity are zeroed at the beginning of a chromatography run. Calibration of the pH detector is described below.

7. Responsibilities

7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials

8.1. AKTA pure chromatography system

8.2. HiTrap AVB Sepahrose HP, Cytiva, Catalog # 28411211 (1 ml) column; stored at 4ºC- bring to room temperature prior to installation.

8.3. Additional Lab Equipment: pH meter, balance, table top centrifuge with swinging bucket rotor

8.4. Lab glassware: Beakers (250, 500ml, 1200 ml), 1 liter and 500 ml graduated cylinders

8.5. Reagents:

8.5.1. Tris base [tris(hydroxymethyl)aminomethane], Fisher bioreagents, catalog# BP152-500

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8.5.2. Sodium Chloride for molecular biology, DNase, RNAse, and Protease free, Acros Organics, Code:327300025
8.5.3. Citric acid, Fisher Scientific, catalog# A-106
8.5.4. L-Arginine hydrochloride, Acros Organics, Code: 105001000
8.5.5. 5N HCl
8.5.6. Filtered deionized water (MilliQ or similar).
8.5.7. Filtered and degassed deionized water (MilliQ or similar)
8.5.8. 20% Ethanol sterile filtered and degassed
8.5.9. 10N NaOH
8.5.10. pH Standard buffers: pH 7, pH 4.01

8.6. Lab Supplies:
8.6.1. Syringe Filters and bottle-top filter system (0.20 µm, and 0.45 µm)
8.6.2. Corning bottles for vacuum filtration, degassing of all chromatography buffers.
8.6.3. Syringes with Leur lock (various sizes- 30 ml for pump system rinse, 3- 10 ml for pH probe calibration on AKTA, 3-10ml for purging and priming the inlets and pump heads, 2-10ml for sample filtering and 1-10 ml for sample injection)
8.6.4. Fraction collection tubes – (30)
8.6.5. Graduated cylinders: 1L, 250 ml, 100 ml
8.6.6. Various sizes of glass beaker (buffer prep, pH electrode calibration)

9. Procedure

9.1. ***Reagent Preparation***:
9.1.1. Buffer A: Binding buffer: 20mM Tris + 200mM NaCl pH 7.8
9.1.1.1. Weigh 1.214g ± 0.02g Tris Base and transfer to a 600 ml beaker with a magnetic stir bar.
9.1.1.2.Weigh 5.845g ± 0.02g Sodium Chloride and transfer to the same beaker.
9.1.1.3.Measure 350 ml MilliQ water in a graduated cylinder and add the water to the solids in the beaker.
9.1.1.4.Stir until the solids have dissolved, check the pH and adjust the pH to 7.8 with 5N HCl. (pH meter should be calibrated immediately prior)
9.1.1.5.Transfer to a 500ml graduated cylinder and adjust the final volume to 500ml.
9.1.1.6. Sterile filter the solution using vacuum, allowing it to degas for 15-20 minutes. Label appropriately.
9.1.2. Buffer B: Elution buffer: 50 mM Citrate, 500mM NaCl, 500 mM Arginine, pH 3.5
9.1.2.1.Weigh 2.88g Citric Acid in a 400 ml beaker with a magnetic stir bar.
9.1.2.2.Weigh 8.76g Sodium Chloride and transfer to the same beaker containing the Citric Acid
9.1.2.3.Weigh 31.606g L-Arginine Hydrochloride and transfer to the beaker used in step 9.1.2.2.
9.1.2.4. Add 210 ml MilliQ water and stir to dissolve solids.

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9.1.2.5. Using a pH meter adjust the pH dropwise with 10N NaOH, to a final pH of 3.5

9.1.2.6. Transfer the solution to a 500 ml graduated cylinder. Adjust the final volume to 300 ml.

9.1.2.7.Sterile filter the solution using vacuum, allowing it to degas for 15-20 minutes. Label appropriately.

9.1.3. 1M Tris base pH 9.00: added to fraction collector tubes to rapidly neutralize acid-eluted fractions from the Protein A column.

9.1.3.1.Weigh 12.11g Tris Base into a plastic weigh boat and transfer to a 200 ml beaker with a magnetic stir bar.

9.1.3.2. Measure 90 ml MilliQ water in a graduated cylinder and transfer to the beaker containing the Tris powder. Stir until dissolved, then adjust pH to 9.0 using a pH meter and 5N, 3N and 1N HCl.

9.1.3.3. Transfer the Tris solution to a 100 ml graduated cylinder, rinsing the beaker with small aliquots of water, which are then added to the cylinder until a final volume of 100 ml is obtained.

9.1.3.4. Filter the solution. Degassing is not necessary.

9.2. ***Start-up and preparation of AKTA pure Instrument and computer***:

Sterile filtered and degassed buffers should be in place prior to turning on the AKTA pure instrument. Equipment start-up requires turning on the instrument and, separately, the computer connected to it.

9.2.1. Place the degassed buffers A and B on top of the AKTA pure instrument.

9.2.2. Locate Inlet tubing A1, A2 and B1, B2 (atop the instrument and resting in water, (short term storage) or 20% ethanol (long term storage). The system if in 20% ethanol the lines to be used should be flushed with water before switching to a buffer containing salts. Inlets A1and B1 each have a filter unit attached, which distinguishes them from A2 and B2; which end in a male threaded fitting and will not be used for a two-buffer procedure.

9.2.3. Rinse tubing Inlet A1 with water and transfer the inlet to the water bottle if it was in 20% ethanol.

9.2.4. Rinse tubing Inlet B1 with water and transfer the inlet to the water if it was in 20% ethanol.

9.2.5. The On/Off switch for the instrument is located on the right side toward the rear of the housing. Switch to the ‘On’ position. Audible emanations from within the instrument cabinet indicate that the AKTA pure system is going through its brief initialization sequence.

9.2.6. The computer On/Off switch is located on the front of the Dell desktop computer unit, near the top of the case. Press in to turn on the computer.

9.2.7. Login to the computer using credentials provided by the College.

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9.2.8. Double click the Unicorn 6.3 icon on the desktop to open the software which controls the instrument functions. Click OK in the "Log In – Unicorn" dialog box that appears.

9.2.9. Open the System Control window (under Tools menu, if not opened automatically on startup).

9.2.10. The top pane of the window will show the current state of the instrument, and the bottom pane shows the fluid path and manual controls. If the window is blank, go to the System menu and select Connect to Systems, check the box by AKTA pure 25 and click OK.

9.3. ***P*riming the pump rinsing system.**

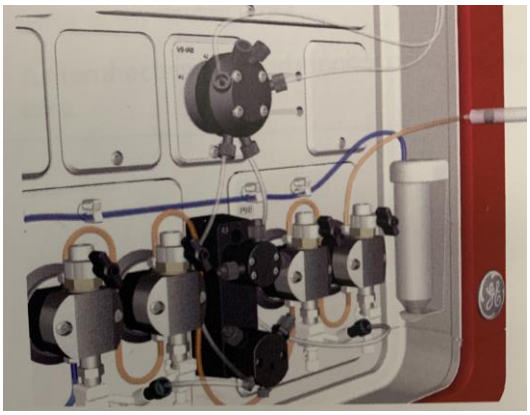
The pump piston rinsing system protects the seal that prevents leakage between the pump chamber and the drive mechanism of the pump. Priming of the pump rinsing system is done before the run

9.3.1. Remove the pump rinsing liquid tube from the holder located on the right-hand bottom corner of the system.

9.3.2. Fill the pump rinsing liquid tube with 50ml of 20% ethanol

9.3.3. Place the pump rinsing liquid tube back in the holder

9.3.4. Insert the inlet tubing to the system pump piston rinsing system in the rinsing solution tube. The inlet tubing is the tubing that runs across the top of the pump heads colored blue in this picture.



(Note: Make sure that the inlet tubing reaches close to the bottom of the rinsing solution tube)

9.3.5. Connect a 25 to 30 ml syringe to the outlet tubing of the system pump piston rinsing system. Draw liquid slowly into the syringe.

9.3.6. Disconnect the syringe and discard its contents.

9.3.7. Fill the rinsing solution tube so that the tube contains 50 ml 0f 20% ethanol.

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9.4. **Prime inlets and purge pump heads.** Before using the system pumps it is important to: Prime the inlets (fill the buffer inlets with liquid) and to Purge the system pumps (remove air from the pump heads)

9.4.1. **Prime the Inlets**.

9.4.1.1. Make sure that all inlet tubing that is to be used during the method run is placed in the correct buffer.

9.4.1.2.Turn on the AKTA pure system if not already on.

9.4.1.3.Open the unicorn 6.3 software

9.4.1.4.Open the **system control** module in the unicorn 6.3 software

9.4.1.5. Fill each of the inlet tubing to be used

9.4.1.5.1. In the **Process Picture** click on the buffer inlets

9.4.1.5.2. Select the position of one of the inlets to be filled. Select the positions in reverse alphabetical order and start with the highest number. For example, if all four inlets are to be filled, fill them in the following order: B2, B1, A2, and A1. The inlet valve switches to the selected port.

9.4.1.5.3. Connect 10 ml syringe to the purge valve of the pump head of the pump that is being prepared. For example, when priming inlet B1 select left pump head of the pump system B. Make sure that the syringe fits tightly.

9.4.1.5.4. Open the purge valve by turning it counterclockwise about three-quarters of a turn. Draw liquid slowly into the syringe until the liquid reaches the pump and no air bubbles are visible in the line.

9.4.1.5.5. Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.

9.4.1.5.6. Repeat step 9.6.1.6.to 9.6.1.9. for each piece of inlet tubing and their respective pump heads.

9.4.2. **Purge System pump B**

9.4.2.1.Make sure that the piece of waste tubing connected to the injection valve port W1 is placed in a waste vessel.

9.4.2.2.In the **Process Picture** click on the injection valve and select **System pump waste**. The injection valve switches to waste position. This is necessary to achieve a low back pressure during purge procedure.

9.4.2.3. In the **Process Picture** click on the **pumps**

9.4.2.4.Set **Conc % B** to 100% B and click **Set % B**. only system pump B is active

9.4.2.5.In the **Process Picture** click on the buffer inlets and select one of the inlets to be used during the run. The inlet valve switches to the selected port.

9.4.2.6.In the **Process Picture** click on the pumps

9.4.2.7. Set the **System flow** to 1.0 ml/min. Click **Set flow rate**. A system flow starts

9.4.2.8. Connect a 10 ml syringe to the purge valve of the left pump head of system pump B. Make sure that the syringe fits tightly into the purge connector.

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9.4.2.9. Open the purge valve by turning it counterclockwise about three quarters of a turn. Draw a small volume of liquid slowly into syringe.

9.4.2.10. Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.

9.4.2.11. Connect the syringe to the purge valve on the right pump head of System pump B, and repeat step 9.4.2.8 to 9.4.2.10. Keep the system flow running.

9.4.3. **Validate purge of pump B**

9.4.3.1. In the **Process Picture** click on the **Injection valve** and select **Manual Load**.

9.4.3.2. Make sure the pump flow is on.

9.4.3.3. In the **Chromatogram** pane check the **PreC pressure**. If the PreC pressure does not stabilize within a few minutes, there may be air left in the pump. Refer AKTA pure system handbook for a troubleshooting guide.

9.4.4. **Purge System pump A**

9.4.4.1. In the **Process Picture** click on the **injection valve** and select **System pump waste.** The injection valve switches to waste position. This is necessary to achieve a low back pressure during purge procedure**.**

9.4.4.2. In the **Process Picture** click on the pumps

9.4.4.3. Set **Conc % B** to 0% B and click **Set % B.** only system pump A is active

9.4.4.4. In the **Process Picture** click on the buffer inlets and select one of the inlets to be used. The inlet valve switches to the selected port.

9.4.4.5. In the **Process Picture** click on the pumps

9.4.4.6. Set the **System flow** to 1.0 ml/min. Click **Set flow rate**. A system flow starts

9.4.4.7. Connect a 10 ml syringe to the purge valve of the left pump head of system pump A. Make sure that the syringe fits tightly into the purge connector.

9.4.4.8. Open the purge valve by turning it counterclockwise about three quarters of a turn. Draw a small volume of liquid slowly into syringe.

9.4.4.9. Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.

9.4.5. Connect the syringe to the purge valve on the right pump head of System pump A, and repeat step 9.4.4.7 to 9.4.4.9. Keep the system flow running

9.4.6. **Validate purge of pump A**

9.4.6.1. In the **Process Picture** click on the **Injection valve** and select **Manual Load**.

9.4.6.2. Make sure the pump flow is on.

9.4.6.3. In the **Chromatogram** pane check the **PreC pressure**. If the PreC pressure does not stabilize within a few minutes there may be air left in the pump. Refer AKTA pure system handbook for a troubleshooting guide.

9.4.7. If the appropriate column is already installed go to step 9.6.(Preparation and installation of super loop)

9.5. **Installing/Changing a Chromatography Column on the AKTA pure Chromatography System**

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It is imperative that the following operations be performed in such a way as to prevent the introduction of air bubbles into the column and fluid path, which is achieved by making liquid-to-liquid (drop-to-drop) contact prior to inserting the threaded fitting into its position.

9.5.1. Have on hand a few paper lab towels and a 250 ml beaker to catch waste.

9.5.2. Initiate flow manually at 0.5 ml/min collecting waste in the beaker or towel.

9.5.2.1. In the Process Picture click on the Pumps.

9.5.2.2.Enter a flow rate of 0.5 ml/min and click set flow rate.

9.5.3. Remove tube connector from the UV detector inlet by unscrewing the knurled fastener. Allow a few drops of the buffer, water or 20% ethanol that is to be used to prepare the column to drip into the UV detector inlet.

9.5.4. Remove the plug from the column inlet and allow a few drops of the water, buffer or 20% ethanol to fill the column inlet, to ensure the absence of air. Also add drops of 20% ethanol to the UV detector inlet.

9.5.5. As a droplet emerges from the inlet tubing, touch it to the liquid in the column inlet and begin to thread the fitting in, leaving slight looseness of threads so that liquid escapes around the fitting and pressure buildup in the column is prevented.

9.5.6. Remove the column bottom plug and screw the column directly into the UV detector inlet.

9.5.7. Tighten the column inlet fitting just enough to prevent leaking.

9.5.8. The column is now ready to **equilibrate with water or buffer A** prior to performing a chromatography run.

9.5.9.

9.6. **Preparation and installation of Superloop 10 sample injection device.** The Superloop is stored disassembled and requires assembly and filling with buffer prior to installation on the AKTA pure instrument, as described here. Gloves should be worn during handling of the Superloop parts.

Refer to the manufacturer's instructions provided on the link below.

https://cdn.cytivalifesciences.com/dmm3bwsv3/AssetStream.aspx?mediaformatid=10061&destinationid=10016&assetid=12806

9.6.1. Rinse and dry the entire disassembled Superloop parts if not done already.

9.6.2. Connect the tubing to the inner pieces

9.6.2.1. Insert the 18 cm tube into a female threaded connector (black).

9.6.2.2. Connect the male threaded connector to the female threaded connector inserted into an 18 cm tubing. Make sure the tube is snug tight.

9.6.2.3. Connect the male threaded connector to one of the inner end pieces.

9.6.2.4. Insert the 28 cm tube into a female threaded connector (black).

9.6.2.5. Connect the male threaded connector to the female threaded connector inserted into a 28 cm tubing. Make sure the tube is snug tight

9.6.3. Rinse/wet O-rings on the end pieces and movable seal with deionized water.

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9.6.4. Insert the movable seal into the graduated glass tube from the bottom (zero) end in such a way that the end with O-ring is closest to the bottom. Using a glass rod with smooth end or a plastic pipette, push the seal into the tube until the O-ring is between the 1ml and 2 ml graduations.

9.6.5. Mount the glass tube on a lab stand with clamp. Working over a sink or container to catch any overfill, pipet enough buffer A into the upper portion of the tube to fill it.

9.6.6. Mind the liquid that will squirt from the tubing; direct it into the sink. Insert the inner end pieces with the 18 cm tubing attached into the glass tube, contacting the liquid meniscus to eliminate air bubble entrapment. Press the end piece completely into the glass tube.

9.6.7. Invert the tube in the clamp/support and wet the movable seal with a small amount of 20% EtOH (or buffer A if it contains a detergent). It may be necessary to use the pipet to eject any air bubbles that stubbornly adhere to the glass and/or movable seal. When bubbles have been eliminated, completely fill the tube with buffer A. Minding the liquid that will squirt from the tubing, insert the remaining inner end piece with tubing attached.

9.6.8. Rotate the bottom inner end piece with 28 cm tubing so that the slotted end (inside the glass tube) aligns with the small notch inside the glass tube. This alignment is important to establish and maintain; otherwise, backpressure in the pumps could increase and prevent completion of the run.

9.6.9. Remove the glass tube with end pieces from the clamp.

9.6.10. Attach the bottom outer end piece by threading it onto the glass tube.

9.6.11. Slide the plastic protective jacket over the glass tube and seat it firmly into the bottom outer end piece.

9.6.12. Attach the top outer end piece to the remaining exposed threaded end of the glass tube.

9.6.13. To install the assembled Superloop 10 onto the AKTA pure instrument, place the lab support and clamp near the instrument on the left side, then mount the Superloop in the clamp. Adjust clamp vertically and horizontally as needed to place the Superloop in close proximity to the injection valve.

9.6.14. Attach the tubing on the top of the Superloop to the injection valve port labeled ‘loop E’ using the threaded connector. Confirm that the tubing is firmly attached and will not easily pull out of the fitting.

9.6.15. By default, the injection valve should be in the ‘Manual Load’ position upon booting up the instrument. Using the manual control feature in the Unicorn software, confirm that the valve is in Manual Load position. If not, switch the valve to the correct position by clicking the injection valve on the system control diagram, then selecting ‘Manual Load’.

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9.6.16. Attach the bottom Superloop tubing to the injection valve port labeled 'loop F' using the threaded connector. Confirm that the tubing is firmly attached and will not easily pull out of the fitting.

9.6.17. The Superloop is now ready to be flushed and prepared for sample injection.

9.7. **Filling the Superloop with Buffer A.** This step is important to ensure that the sample chamber and backside of the loop are equilibrated with the starting buffer for a given chromatographic run. Most of the residual ethanol and other compounds in the injection valve flow path will be expelled in the process.

9.7.1.1. Place degassed Buffer A atop the instrument and insert inlet tubing A1 into the buffer container, ensuring that the filter rests on the bottom of the container.

9.7.1.2. Use the pump priming syringe connected to pump A1 to draw 10 ml of buffer A through the inlet A1 tubing and close the priming valve. Using the manual control panel in the System Control window, click on Pump A in the diagram and select "Pump A Wash".

9.7.1.3. Upon completion of the wash, set the flow rate to 1 ml/min. Click on the injection valve depiction and select "Inject".

9.7.1.4. When the Superloop movable seal arrives at the zero position, change the injection valve position to 'Manual Load'. Allow the pump to continue at 1 ml/min.

9.7.1.5. Fill a 10 ml syringe with buffer A and inject sufficient volume to completely fill the Superloop. See section 9.11.1.9. for more information on sample injection.

9.7.1.6. Once again, change the injection valve position to 'Inject' using the manual control feature of the software interface. Increase the flow rate to 2 ml/min.

9.7.1.7. When the Superloop movable seal is at the zero position, stop the pump. Click the 'Stop' icon (a solid square) in the toolbar near the top of the System Control window.

9.8. **Calibration of the pH Electrode**

Calibration of the pH detector is performed daily, when the instrument is in use. The calibration procedure utilizes ordinary pH standards found in the lab. Calibration is dictated by a method built into Unicorn.

9.8.1. Obtain three small beakers and pH standards for pH 4.01 and pH 7.0, as well as a 10 ml syringe and a bottle of MilliQ water.

9.8.2. In the Unicorn System Control window, choose 'Calibration' from the System menu. From the drop down, menu under 'Monitor to calibrate', select 'pH'.

9.8.3. Click the 'Prepare for Calibration' button. You will hear the valve switch to the calibrate position.

9.8.4. Follow the on-screen instructions for both pH standards. Enter the pH of the first pH standard buffer in the ***pH for buffer 1*** field.

9.8.5. Fill a syringe with approximately 10 ml of the first pH standard buffer (pH 7). Connect the syringe to the Luer connector of pH valve port **Cal**, and inject the buffer. When the ***Current value*** is stable, click the ***Calibrate*** button.

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9.8.6. Thoroughly rinse the syringe with 3-4 changes of MilliQ water. Wash the pH flow cell by injecting water into pH valve port **Cal**.

9.8.7. Enter the pH of the second pH standard buffer in the ***pH for buffer 2*** field. Fill a syringe with approximately 10 ml of the second pH standard buffer. Connect the syringe to the Luer connector of pH valve port **Cal** and inject the buffer. When the ***Current value*** is stable, click the ***Calibrate*** button.

9.8.8. The calibration date and time are displayed in the dialog, along with values for ***Calibrated electrode slope*** (should be ≥ 80%) and ***Asymmetry potential at pH 7*** (should be within the interval ± 60 mV. If the conditions are met, click the ***Close*** button to switch the pH valve back to the default position and to close the ***Calibration*** dialog.

9.8.9. If values for the slope and potential are not within acceptable values, clean the pH electrode and repeat the calibration procedure. If this does not help, replace the electrode.

9.9. **Preparation of the Fraction Collector**

9.9.1. Prepare 30 fraction collector tube by adding 125µl of 1M Tris pH 9.00 to the bottom of each tube – this serves to rapidly neutralize the acidic eluent (which destabilizes AAV particles).

9.9.2. Load prepared 30 collection tubes into the fraction collector starting at position 1.

9.9.3. Place the fraction collector tube 1 near the outlet tubing from the instrument (refer to attachment Fig 1) so that it will touch the arrow on the white paddle of the fraction collector arm. Note: To rotate the carousel, reach around the left side of the collector to find a rubber roller pressing against the carousel (Fig 2). Pull the roller away from the carousel (Fig. 3); the carousel will rotate freely as long as the roller is held. When the first tube is in the correct position, release the roller.

9.9.4. Gently raise the arm and swing it into position against tube 1.

9.10. **Performing chromatographic run**

Chromatographic run sequence summary:

A. Column Equilibration run sequence summary:

1. *Prepare the column with 1 Column Volume of 100% buffer B (Elution buffer); flowrate 1.0ml/min*
2. *Equilibrate the column with 5 Column Volume of 100% buffer A (Binding buffer); flowrate 1 ml//min*

B. Run Sequence Summary

1. *Inject 9.5ml crude material from the superloop; begin collecting 2.5ml fractions; flow rate 0.5ml/min*
2. *Wash unbound proteins through with up to 15 column volume (CV) 100% buffer A, until A280 stabilizes at baseline; collecting 2.5ml fractions. Flow rate 1 ml/min*
3. *Elute bound AAV viral particles with step to 100% buffer B; collecting 1 ml fractions and 1 ml peak fraction, for a total of 15 CV*

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4. *Re-equilibrate column in buffer A until pH stabilizes; maximum 20 CV. Eluent to waste*

Degassed Buffer A and Buffer B should be in place prior to turning on the AKTA pure instrument. Equipment start-up requires turning on the instrument and, separately, the computer connected to it.

9.10.1. Locate Inlet tubing A1 and B1 (atop the instrument and resting in water or 20% ethanol). Each has a filter unit attached, which distinguishes them from A2 and B2; those end in a male threaded fitting and will not be used for a two-buffer procedure.

9.10.2. Transfer tubing Inlet A1 to the buffer A bottle. (If not already in buffer A)

9.10.3. Transfer tubing Inlet B1 to the buffer B bottle. (if not already in buffer B)

9.10.4. Check that the volumes of Buffer A and Buffer B are more than sufficient to complete the run.

9.10.5. Confirm that all "Waste" tubing, labeled W, W1 and W2 in a 1L Erlenmeyer flask, and that the tube labeled "Out" is in a 125ml Erlenmeyer flask.

9.10.6. Confirm that the " HiTrap AVB Sepharose HP" (1 ml) column is attached to the system and appropriately prepared. If not, refer to Section 9.5. (Installing/Changing a Chromatography Column on the AKTA pure Chromatography System).

9.10.7. Confirm that the Superloop is installed.

9.10.8. Confirm that the fraction collector is ready.

9.11. **Run a Programmed Method**

9.11.1.1. In the Unicorn Software navigate to the System Control window. Under the File menu choose open and select the method with file name "1 ml AVB Sepharose Equilibration"

9.11.1.2. In the dialog box that opens; Click Next; note the time and volume for the run; make sure there is excess buffer A and B.

9.11.1.3. Click Next. Record the buffer composition of each buffer and the sample identity.

9.11.1.4. Click Next. Enter a filename composed of the method name, date, operator or group initial.

9.11.1.5. Click Start. The instrument should begin to execute the method, as evidenced by a soothing hum from the pumps and liquid dropping in the waste container

9.11.1.6. Monitor the computer screen for error messages or warnings.

9.11.1.7. Allow the method to run to completion, about 15 minutes

9.11.1.8. Fill a 10 ml syringe with the 0.45 µm syringe filtered HEK293 AAV GFP cell lysate sample, expel any bubbles and insert the loaded syringe into the injection port.

9.11.1.9. Inject the sample into the port to fill the 10ml Superloop.

9.11.1.10. Open the Unicorn software and navigate to the System Control window.

9.11.1.11. Under the File menu, choose Open and select the method with file name "1ml AVB Sepharose HP Column".

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9.11.1.12. In the dialog box that opens, enter operator's name, sample notes.
9.11.1.13. Click Next; take note of the time and volume for the run; make sure there is excess buffer A and B.
9.11.1.14. Click Next. Enter the buffer composition of each buffer and the sample identity.
9.11.1.15. Click Next. Enter a filename composed of the method name, date, operator or group initials, for example **HiTrap AVB AAV (15MAY23**) SD Biopharm.
9.11.1.16. Click Start. The instrument should begin to execute the method, as evidenced by a soothing hum from the pumps and liquid dropping in the waste container
9.11.1.17. Monitor the computer screen for error messages or warning
9.11.1.18. Allow the method to run to completion, about 45 minutes

9.12. ***Equipment shut-down and short term (less than 3 days) storage***

9.12.1. After completion of the final separation of the day, transfer Inlet tubing A1 and B1 to a flask of degassed Milli-Q water (250 ml or greater).
9.12.2. In the Unicorn software, open the System Control window.
9.12.3. Under the File menu, choose Open, then select the method 'System Short Term Storage'.
9.12.4. Click Start.
9.12.5. Allow the method to run to completion, as indicated by an audible tone and onscreen window.
9.12.6. Place the pH valve in the 'Calibration' position (System Control window; System\Calibrate menu). Fill a 10 ml syringe with pH electrode storage solution and inject 9 ml into the calibration port. Leave the syringe attached.

9.13. Turn off the instrument or perform the long-term storage routine as needed (section 9.15).

9.14. ***Cleaning of the Superloop 10 sample holder- short term.***

9.14.1. For short term storage of the Superloop on the AKTA instrument, inject 2 ml Milli-Q water into the sample chamber.
9.14.2. Pump it out to waste by temporarily disconnecting the outlet tubing that is connected to the injection valve at port 'loop F'.
9.14.3. Using manual control in the System Control window of Unicorn, set the flow rate to 2 ml/min and the injection valve position to Inject. Allow pump A to run until the Superloop chamber is empty.
9.14.4. Reconnect to 'loop F'.
9.14.5. Repeat steps 9.14.1 thru 9.14.4 three times.
9.14.6. Inject 10 ml water into the sample chamber of the Superloop.

9.15. ***Equipment shut-down and long term (3 days or more) storage***

9.15.1. After completion of the System Short Term Storage method, transfer Inlet tubing A1 and B1 to a flask of degassed 20% ethanol (250 ml or greater).
9.15.2. In the Unicorn software, open the System Control window.

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9.15.3. Confirm that the pH valve is in the 'Bypass' or 'Restrictor' position.
9.15.4. Under the File menu, choose Open, then select the method 'System Long Term Storage'.
9.15.5. Click Start.
9.15.6. Allow the method to run to completion, as indicated by an audible tone and onscreen window.
9.15.7. Turn off the instrument.
9.15.8. Remove the Superloop from the instrument and carefully disassemble it. Hand wash all parts with a general purpose lab cleaner, rinse well and allow to air dry. Store the dried components in their original box.

9.16. ***Cleaning the system***

9.16.1. Column Removal – have paper towels on hand to catch drips. You will also need an appropriate plug for each end of the column.
9.16.1.1. Use the Manual Control in the System Control window to set the flow rate of buffer or water to 0.5 ml/min.
9.16.1.2. Disconnect the column outlet from the UV detector and fit the connector with a plug, slowly so that emerging droplets displace *all* air from the plug. Leave the plug slightly loose so as to prevent backpressure buildup.
9.16.1.3. Slowly disconnect the tubing from the column inlet, allowing liquid to fill the cavity where the connector attaches. Attach the tubing directly to the UV detector inlet and stop the flow of buffer/water/20% ethanol.
9.16.1.4. Completely tighten the column outlet plug, but be careful not to overtighten and strip the threads.
9.16.1.5. Carefully insert a plug into the column inlet threads, displacing liquid but not allowing air to enter.
9.16.1.6. The column may now be stored.
9.16.2. Minimal Cleaning – After every day of use, perform short-term storage (9.18).
9.16.3. Thorough Cleaning – Should be performed weekly.
9.16.3.1. Remove the column from the system prior to thorough cleaning of the system with 0.5M NaOH.
9.16.3.2. Immerse all pump inlet tubes in a container of 0.5M NaOH.
9.16.3.3. **Important:** Switch the pH electrode valve to 'Bypass' or 'Restrictor' position to prevent damage to the electrode.
9.16.3.4. Run the method 'System Clean'.
9.16.3.5. Remove the pump inlet tubing from the NaOH container, rinse each carefully with a squirt bottle of MilliQ water and place in a flask of filtered and degassed MilliQ water.
9.16.3.6. Run the method 'System Short Term Storage'.

9.17. ***Chromatogram printout***

9.17.1. In the Unicorn software interface, open the Evaluation window.
9.17.2. In the Result Navigator pane, click the Results tab.

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9.17.3. Locate the file of interest and double click its name to display your chromatogram in the right pane.

9.17.4. *Optional:* Click the Customize button to open a dialog box that allows you to specifiy what curves display and the scale of each axis. Recommended are the UV Chrom curve, Conductivity, and Fraction Number.

9.17.5. Click the Report button, check the Default report in the selection window and click Preview.

9.17.6. Under File, choose to Print (or Save as PDF to use a different printer).

10. Attachments/Figures

Fig. 1. ***Diagram of AKTA pure instrument, fraction collector and computer***

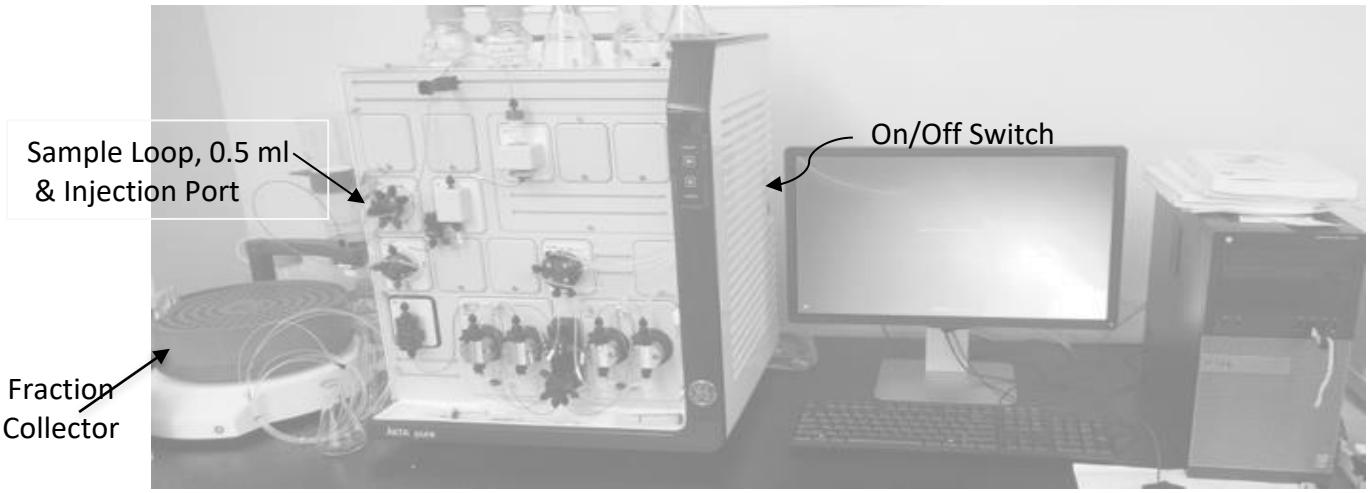

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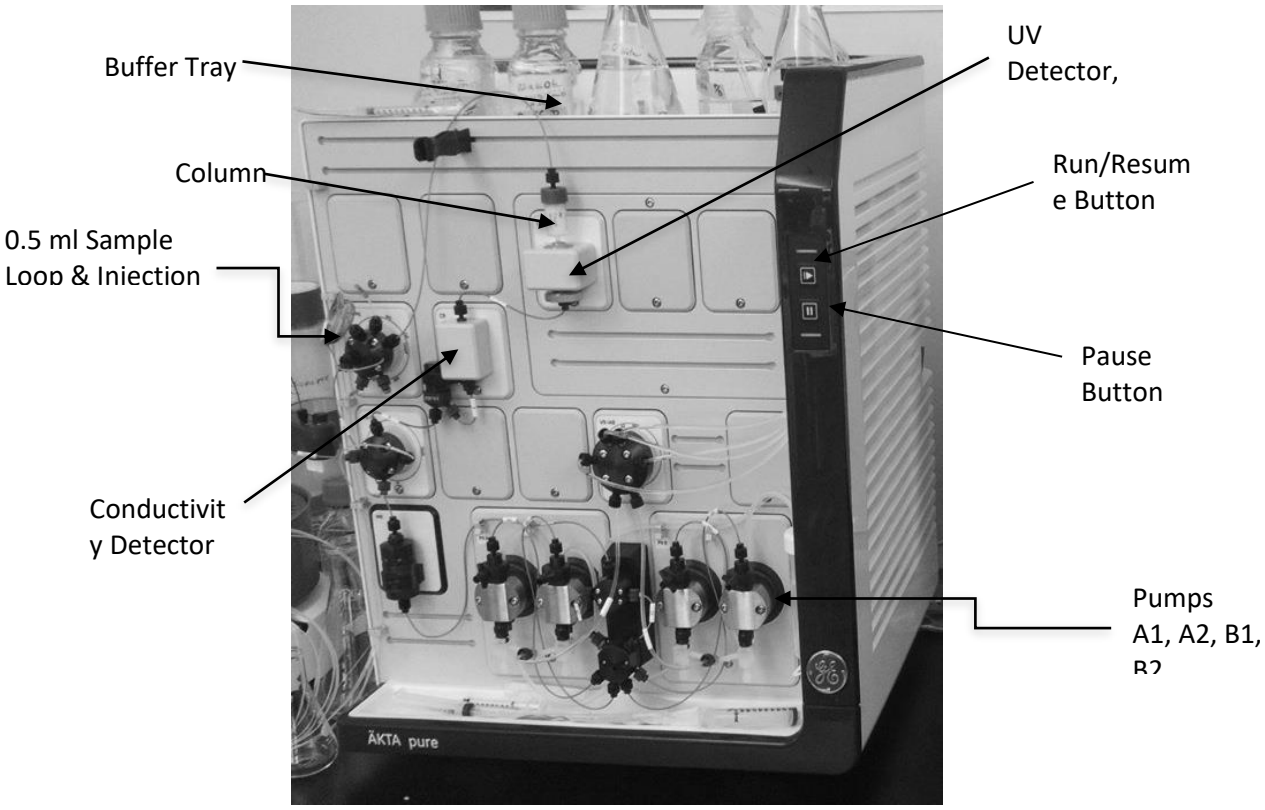
Fig. 2. ***AKTA pure Instrument Features***

Fig 3. ***System Control window within the Unicorn 6.3 software.***



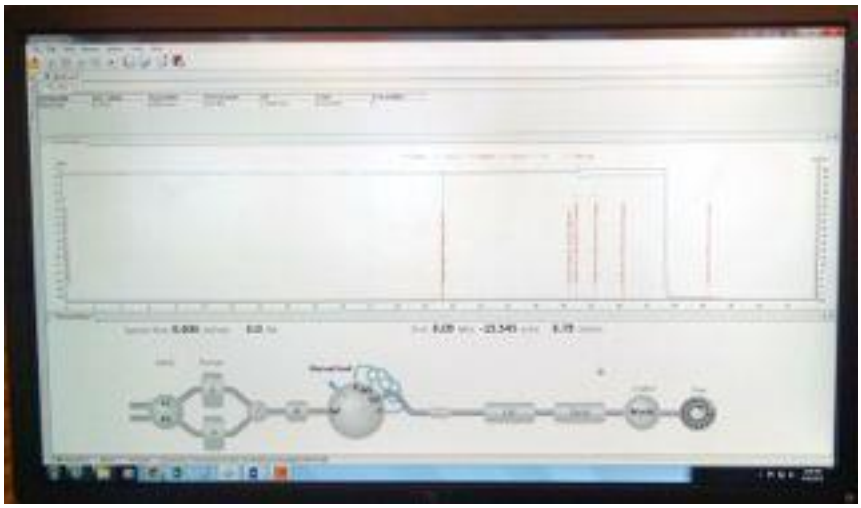


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Fig. 4. ***Detail of Injection Port with Syringe in Place***.

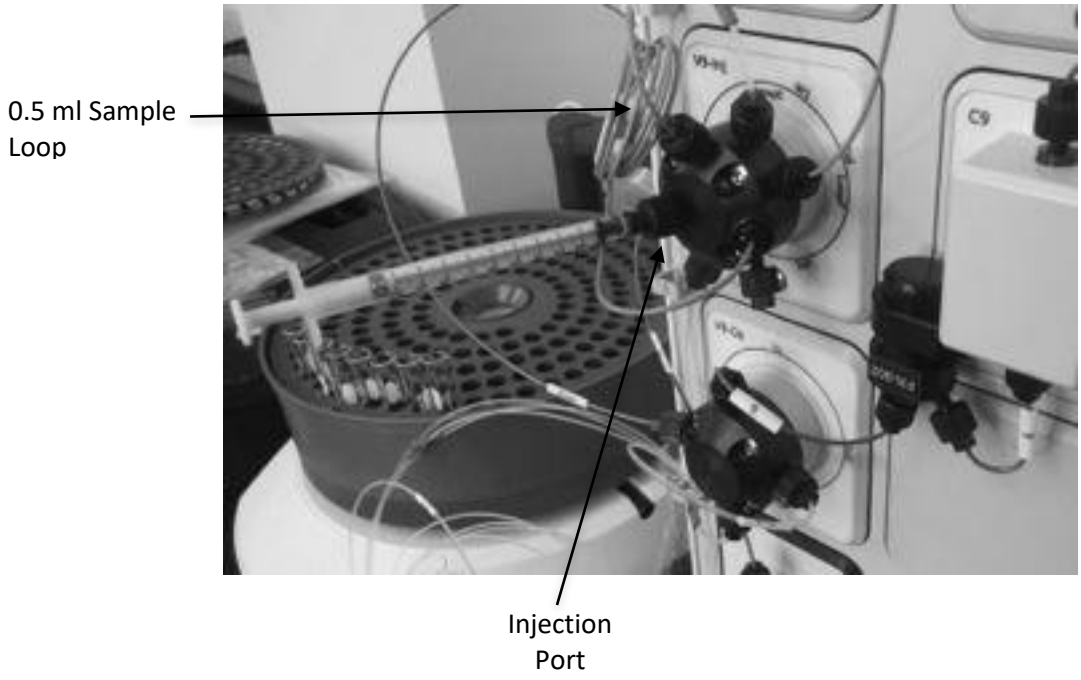

Fig. 5. ***Fraction collector carousel rubber advancement roller/gear.***

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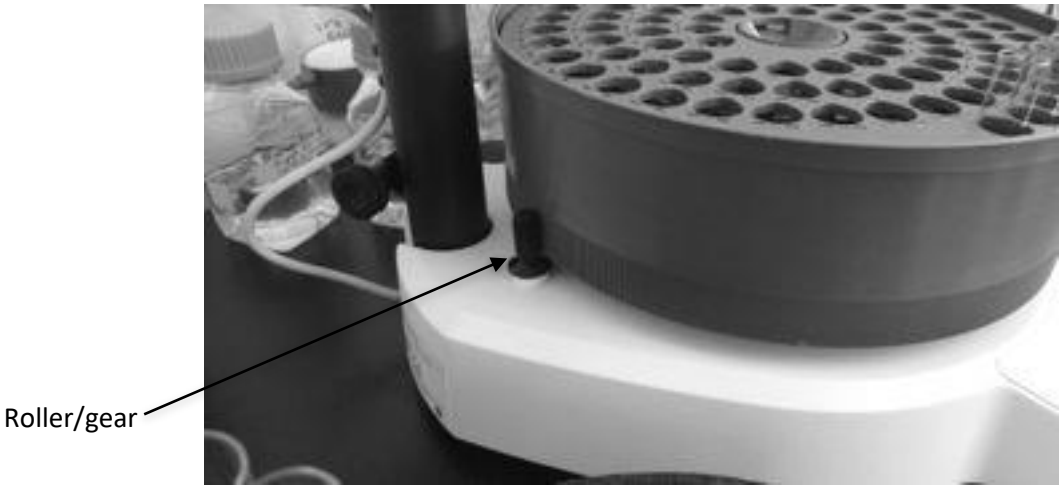

Fig. 6. ***Release of roller to allow free rotation of the carousel.***

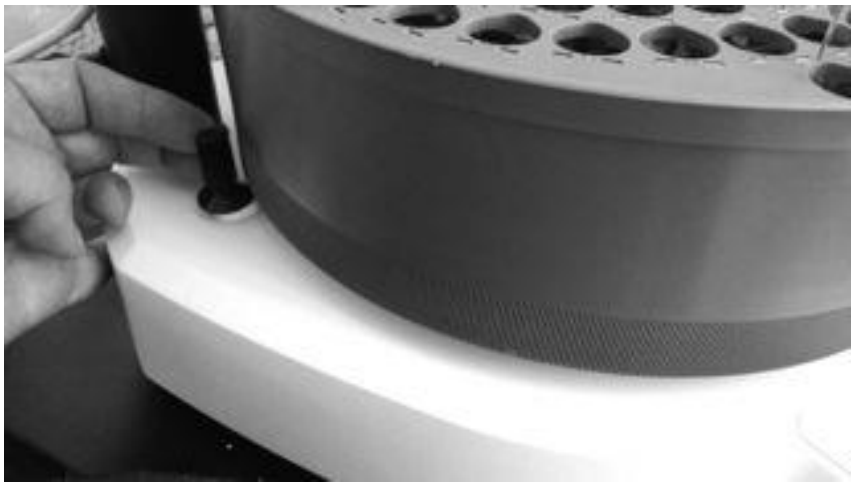

Fig. 7. ***Location of tube #1 under the fraction collector drip outlet.***

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11. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	20MAY23	Hetal Doshi	Initial release