SOP: Anion Exchange Chromatography of Green Fluorescent Protein (GFP) using the AKTA Pure system

Approvals

Preparer: Robin Zuck	Date: 04MAR19
Reviewer: Hetal Doshi	Date: 08MAR19
Reviewer: Dr. Maggie Bryans	Date: 16MAR19

1. Purpose

1.1. This procedure describes the operation of the ÄKTA pure Chromatography System, controlled by Unicorn 6.3 software, for the purpose of anion exchange chromatography of samples containing green fluorescent protein (GFP).

2. Scope and Applicability

2.1. Applies to purification of GFP from diverse origins, including bacterially expressed recombinant GFP. Cell lysates may be prepared by a variety of methods. The prepared lysate sample for purification should have a pH between 7.5 and 8.2. This procedure uses a Hi Trap Capto Q HP 5 ml column installed on the GE ÄKTA pure Chromatography System and controlled by Unicorn 6.3 software.

3. Summary of Method

3.1. Preparation of buffer(s)
3.2. Equilibration of system and column
3.3. Fraction collector setup
3.4. Application of sample
3.5. Washing and elution of column
3.6. Regeneration of system in preparation for subsequent run
3.7. Procedures for 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 (electronic)
4.3. AKTA pure Operating Instructions (Note: Chapter 4 Section 5)
4.4. Hi Trap Capto Q HP 5 information booklet
4.5. SOP: Operation of AKTA Pure Chromatography System

5. Definitions

N/A

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.

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6.3. Avoid damaging the threads through the use of excessive force when connecting plastic fasteners.
6.4. 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.
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.
6.7. Samples should be centrifuged at 10000x*g* for 5 min and then sterile filtered using a 0.2 μm filter before injection/introduction into the fluid path.
6.8. Equipment calibration check: The AKTA pure system calibration of A280 and conductivity are automatic; baseline for measurements of A280 and conductivity are zeroed at the beginning of a chromatography run. However, calibration of the pH detector must be performed prior to use of the instrument each day, using standard calibration buffers and the automated routine in Unicorn. It is assumed that calibration is performed according to the Equipment SOP for the AKTA pure 25 instrument. Further adjustment is beyond the scope of this document and should be referred to a qualified technician.

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. Additional Lab Equipment: pH meter, balance
8.3. Lab Utensils: Beakers (250, 500ml), 500 ml graduated cylinders
8.4. Reagents: Tris, hydrochloric acid, sodium chloride, filtered deionized water (Milli Q or similar), 20% ethanol.
8.5. Lab Supplies: Filters (0.2 μm) and bottles for vacuum filtration and degassing of all chromatography buffers. Syringe (1ml). Syringe filters (0.2 μm). Tubes for fraction collector.

9. Procedure

9.1. ***Sample Collection and Preparation*** is described elsewhere, (for example the SOP: Preparation of Bacterial Cell Lysates using BPER Reagent DP20, 20MAY2019). This chromatography method is also appropriate for samples in compatible buffer such as 50 mM Tris-HCl, pH 8.2. The operator will require 0.6 ml of sample per sample injection (see below).
9.2. ***Reagent Preparation***: Buffers should be prepared dependent on the mode of separation employed; in this instance anion exchange chromatography provides good separation of GFP from contaminating protein at pH 8.2.

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9.2.1. Anion Exchange Buffer A: 50 mM Tris-HCl, pH 8.2

9.2.1.1. Dissolve 6.057 gm Tris in 950 ml filtered deionized water in a one liter beaker, with stir bar.

9.2.1.2. Titrate the pH of the Tris solution to 8.2 by addition of concentrated HCl or 5M HCl, carefully adding the appropriate acid dropwise to obtain pH 8.2.

9.2.1.3. Adjust the final volume to 1000 ml. Set aside 500 ml of this solution and label 'Buffer A' along with its precise composition and date of preparation. Filter and degas the buffer by passage through a vacuum filter device attached to house vacuum, leaving the filtered solution under vacuum for 15-20 minutes.

9.2.1.4. Anion Exchange Buffer B: Use the remaining 500 ml to dissolve 29.22 gm of NaCl in an 800 ml beaker. Following dissolution, filter and degas this mixture and label the bottle 'Buffer B', along with the actual contents (50mM Tris-HCl, pH 8.2, 1 M NaCl).

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

Note: Refer to the AKTA pure Operating Instructions (Note Chapter 4 Section 5), for purging and priming the lines and pumps.

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.

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

9.3.2. 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 this procedure.

9.3.3. If the Inlet tubing A1 and B1 are resting in water;

9.3.3.1.Transfer tubing Inlet A1 to the buffer A bottle.

9.3.3.2.Transfer tubing Inlet B1 to the buffer B bottle.

9.3.4. If the Inlet tubing A1 and Inlet tubing B1 are in 20% ethanol transfer both Inlet tubings to sterile filtered, degassed Milli Q water. The ethanol must be flushed from the system and column before equilibrating the column with the buffers.

9.3.5. 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.

9.3.6. 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.

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

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9.3.8. 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.

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

9.3.10. 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.

9.3.11. Confirm that the correct column (Hi Trap Capto Q 5 ml) is attached to the system. If not, refer to Section 9.4 (Installing/Changing a Chromatography Column on the AKTA pure Chromatography System).

9.3.12. If the Inlet tubing A1 and B1 were transferred from 20% ethanol to water

9.3.12.1. Under the File menu, choose Open and select the method "System Short term Storage".

9.3.12.2. A dialog box appears that allows the method to be run. Click Start to initiate flushing of the pumps and column to remove ethanol.

9.3.12.3. Allow to run to completion.

9.3.12.4. Transfer Inlet tubing A1 to Buffer A.

9.3.12.5. Transfer Inlet tubing B1 to Buffer B.

9.3.13. Prepare the Fraction Collector by filling the carousel with 63 tubes.

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

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

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

9.4.2. Remove tube connector from the UV detector inlet by unscrewing the knurled fastener.

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

9.4.4. Remove the plug from the column inlet and place a few drops of 20% ethanol in the inlet, filling it to insure the absence of air.

9.4.5. 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.

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

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

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9.5. ***Performing a chromatography run***:

9.5.1. 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.

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

9.5.3. Place all 'Waste' tubing, labeled W, W1 & W2 in 1 L Erlenmeyer flask.

9.5.4. Place the tube labeled Outlet in a 125 ml Erlenmeyer flask.

9.5.5. Using a 1 ml syringe flush the injection loop with 1 ml of sterile milli Q water and then with 1 ml of Buffer A.

9.5.6. Using a 1 ml syringe, aspirate 0.6 ml of the GFP sample into the syringe, expel any bubbles and insert the loaded syringe into the injection port.

9.5.7. Inject the sample into the port to fill the 0.5 ml sample loop.

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

9.5.9. Under the File menu, choose Open and select the method with file name "*AE Capto Q 5ml for GFP eq wash step grad"*.

9.5.10. In the dialog box that opens, enter operator's name, sample notes.

9.5.11. Click Next; note the time, (51minutes) and volumes (93ml Buffer A, 33 ml Buffer B), for the run; make sure there is excess Buffer A and B.

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

9.5.13. Click Next. Enter a filename composed of the method name, date, operator or group initials, for example HiTrapQ GFP AEX 16May15 *MyGroupName*.

9.5.14. Click Start. The instrument should begin to execute the method, as evidenced by a soothing hum from the pumps and drops of liquid falling into tube 1 from the fraction collector outlet.

9.5.15. Observe that the fraction collector is receiving drops.

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

9.5.17. Allow the method to run to completion, at which time the system will be re-equilibrated and ready for subsequent runs by repeating section 9.4.

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

9.6.1. After completion of the final separation of the day, transfer Inlet tubing A1 and B1 to a flask of degassed Sterile filtered Milli Q water (250 ml or greater).

9.6.2. In the Unicorn software, open the System Control window.

9.6.3. Under the File menu, choose Open, then select the method 'System Short Term Storage'.

9.6.4. Click Start.

9.6.5. Allow the method to run to completion, as indicated by an audible tone and onscreen window.

9.6.6. Turn off the instrument or perform the long term storage routine as needed (section 9.6).

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

9.7.1. 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).

9.7.2. In the Unicorn software, open the System Control window.

9.7.3. Under the File menu, choose Open, then select the method 'System Long Term Storage'.

9.7.4. Click Start.

9.7.5. Allow the method to run to completion, as indicated by an audible tone and onscreen window.

9.7.6. Turn off the instrument.

9.8. ***Chromatogram printout***

9.8.1. In the Unicorn software interface, open the Evaluation window.

9.8.2. In the Result Navigator pane, click the Results tab.

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

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

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

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

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Attachments/Figures

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

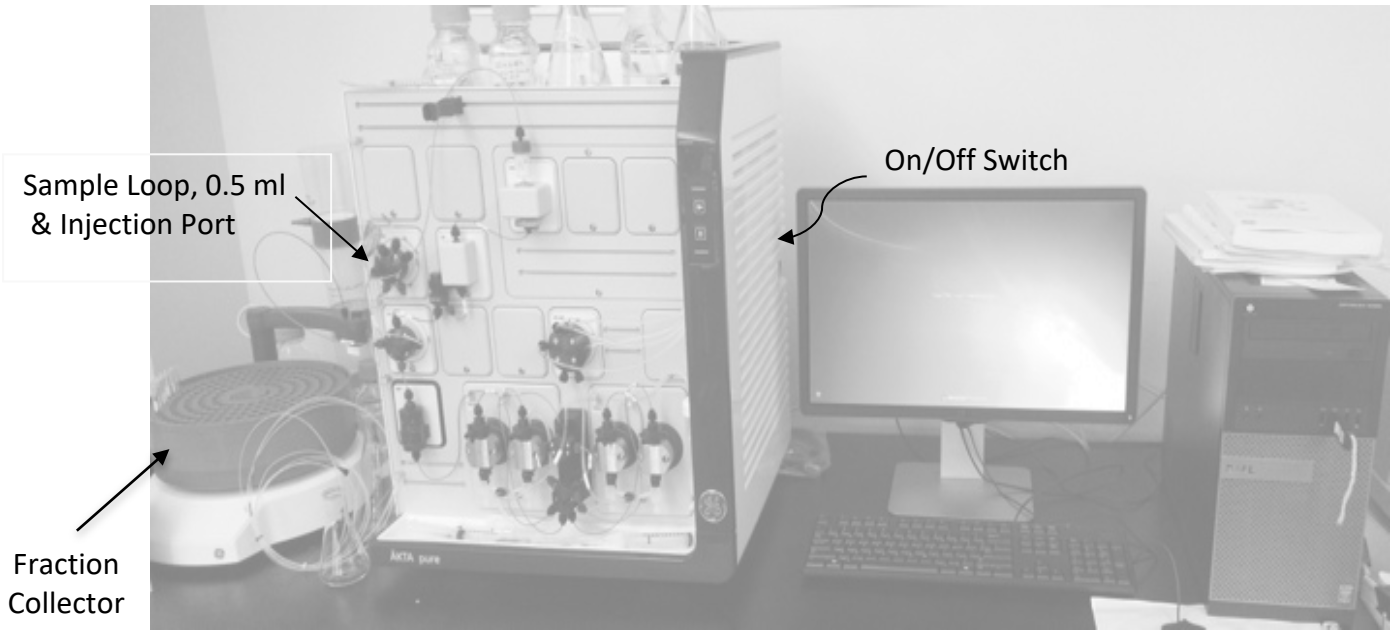

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Fig. 2. ***AKTA pure Instrument Features***

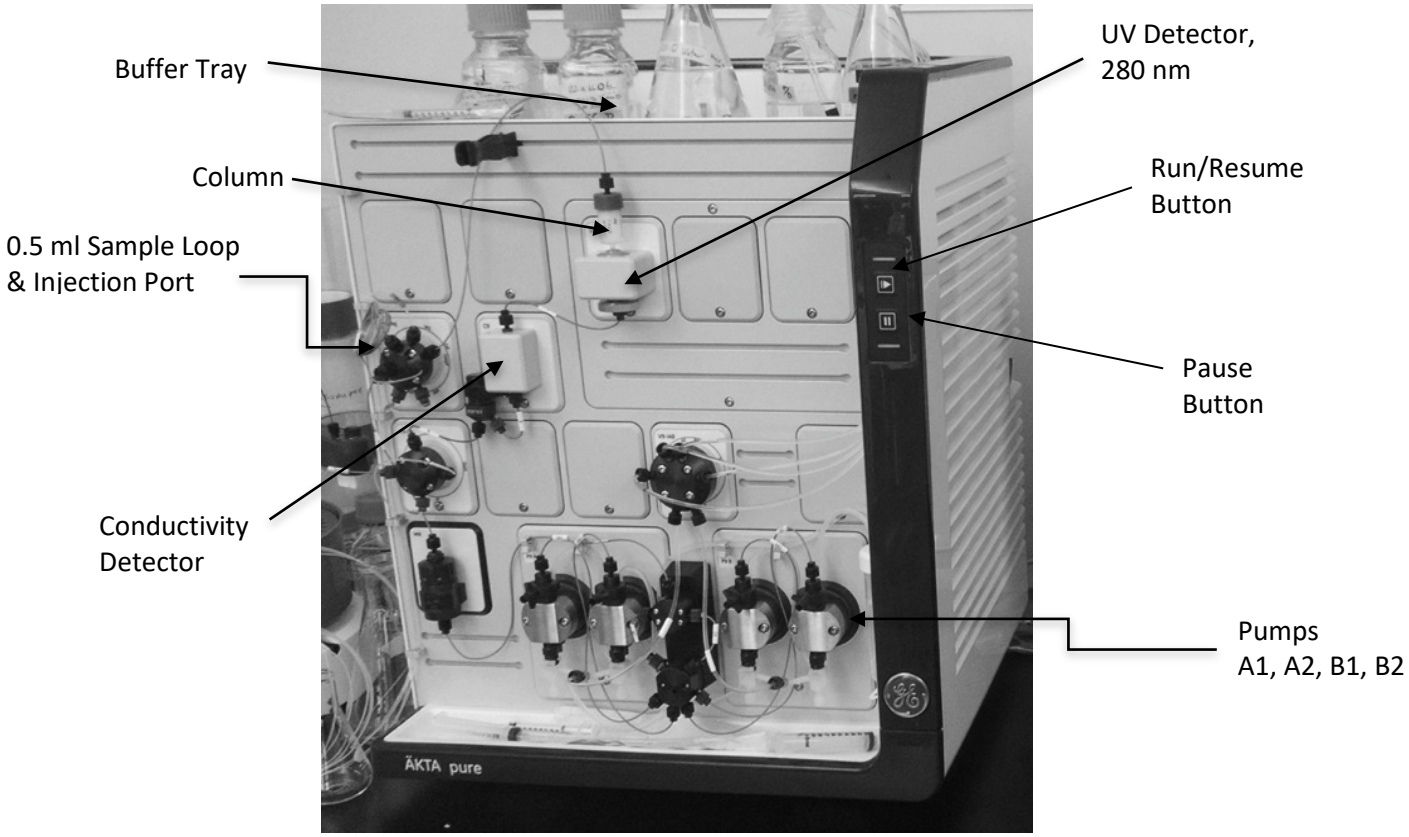

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Fig. 3. ***Detail of Injection Port with Syringe in Place***.

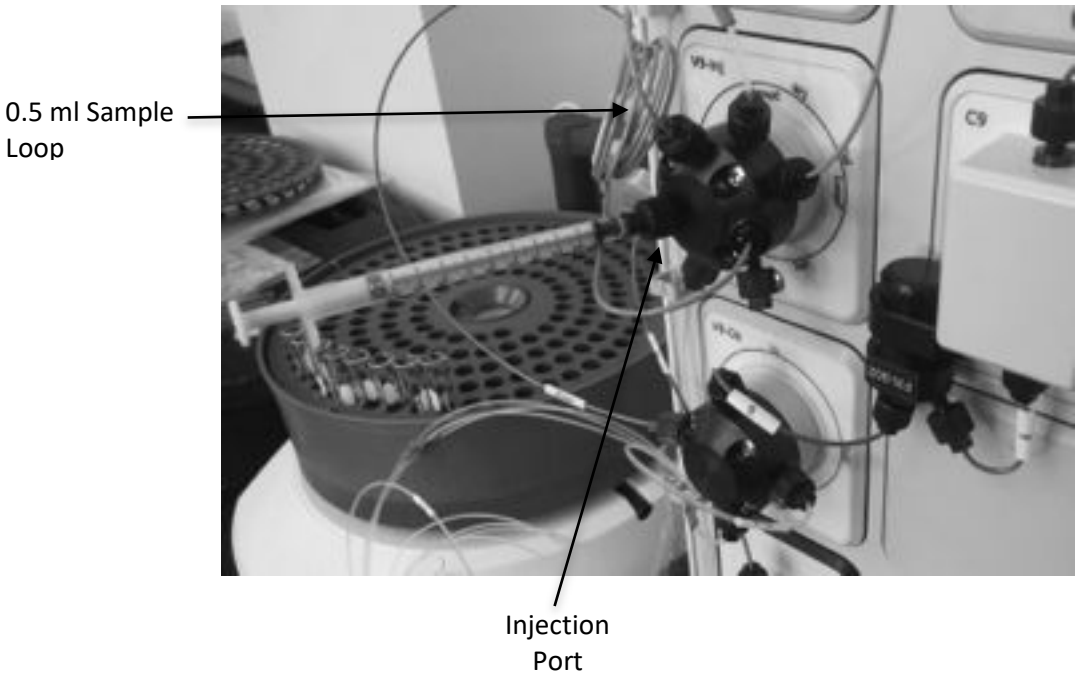

Fig. 4. ***Fraction collector carousel rubber advancement roller/gear.***

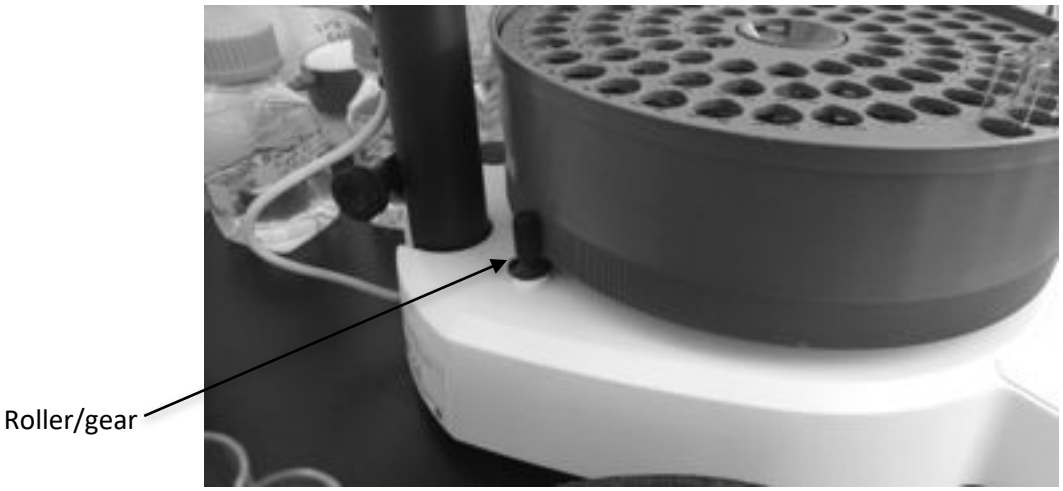

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Fig. 5. ***Release of roller to allow free rotation of the carousel.***

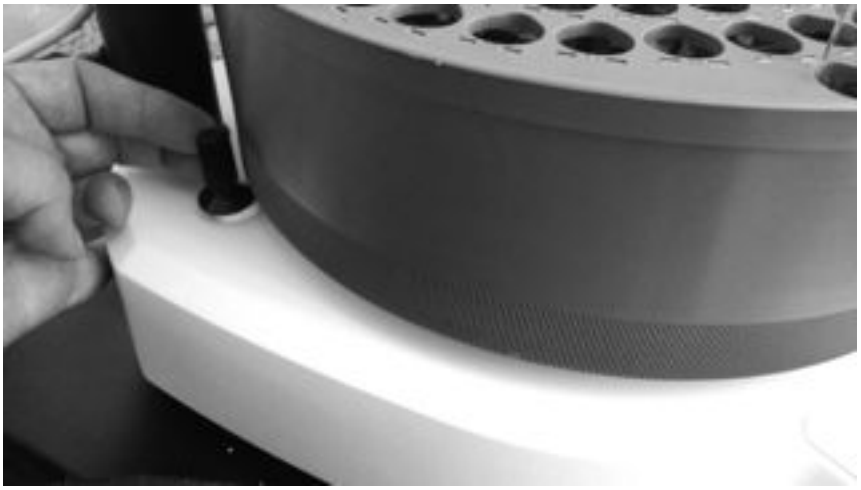

Fig. 6. ***Location of tube #1 under the fraction collector drip outlet.***

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Attachment 1. Programmed method - AE Capto Q 5ml for GFP eq wash step gradient.

Method Settings:

Technique: anion exchange

Column: Hi Trap Capto Q 5ml

Unit Selection: volume

Flow rate: 2.5 ml/min

Inlets A1 and B1

Equilibration:

Reset UV monitor

Flow rate: 2.5 ml/min 3 column volumes

Inlets A1 and B1, 100% A 0% B

Sample Application:

Flow rate 1.00 ml/min

Inlets A1 and B1, 100% A 0% B

Inject sample from loop Capillary loop Empty loop with 1.00 ml

Fractionate Using fraction collector fixed volume fractionation 1.00 ml

Column Wash:

Flow rate 2.5 ml/min 3 column volumes

Inlets A1 and B1, 100% A 0% B

Fractionate using fraction collector fixed volume 2.00 ml

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Elution step 10% B:

Flow rate 2.5 ml/min 6 column volumes

Inlets A1 and B1, 90% A 10% B

Fractionate using fraction collector fixed volume 2.00 ml

Elution step 33% B:

Flow rate 2.5 ml/min 6 column volumes

Inlets A1 and B1, 66.6% A 33.3% B

Fractionate using fraction collector fixed volume 1.00 ml

Elution step 100% B:

Flow rate 2.5 ml/min 4 column volumes

Inlets A1 and B1, 0% A 100% B

Fractionate using fraction collector fixed volume 5.00 ml

Equilibration:

Flow rate 2.5 ml/min 3 column volumes

Inlets A1 and B1, 100% A 0% B

Attachment 2. Programmed method – System Short Term Storage.

Method Settings:

Technique: anion exchange

Flow rate: 5.0 ml/min

Inlets A1 and B1 both in sterile filtered degassed milli Q water

Column Wash:

Flow rate 5.0 ml/min

Inlets A1 and B1, 50% A 50% B

Fill system 15ml

Wash 5 column volumes

Attachment 3. Programmed method – System Long Term Storage.

Method Settings:

Technique: anion exchange

Flow rate: 5.0 ml/min

Inlets A1 and B1 both in sterile filtered degassed 20% ethanol

Column Wash:

Flow rate 5.0 ml/min

Inlets A1 and B1, 50% A 50% B

Fill system 15ml

Wash 2 column volumes

Attachment 4. Programmed method – System Short Term Storage.

Method Settings:

Technique: anion exchange

Flow rate: 5.0 ml/min

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Inlets A1 and B1 both in sterile filtered degassed Milli Q water.

Column Wash:

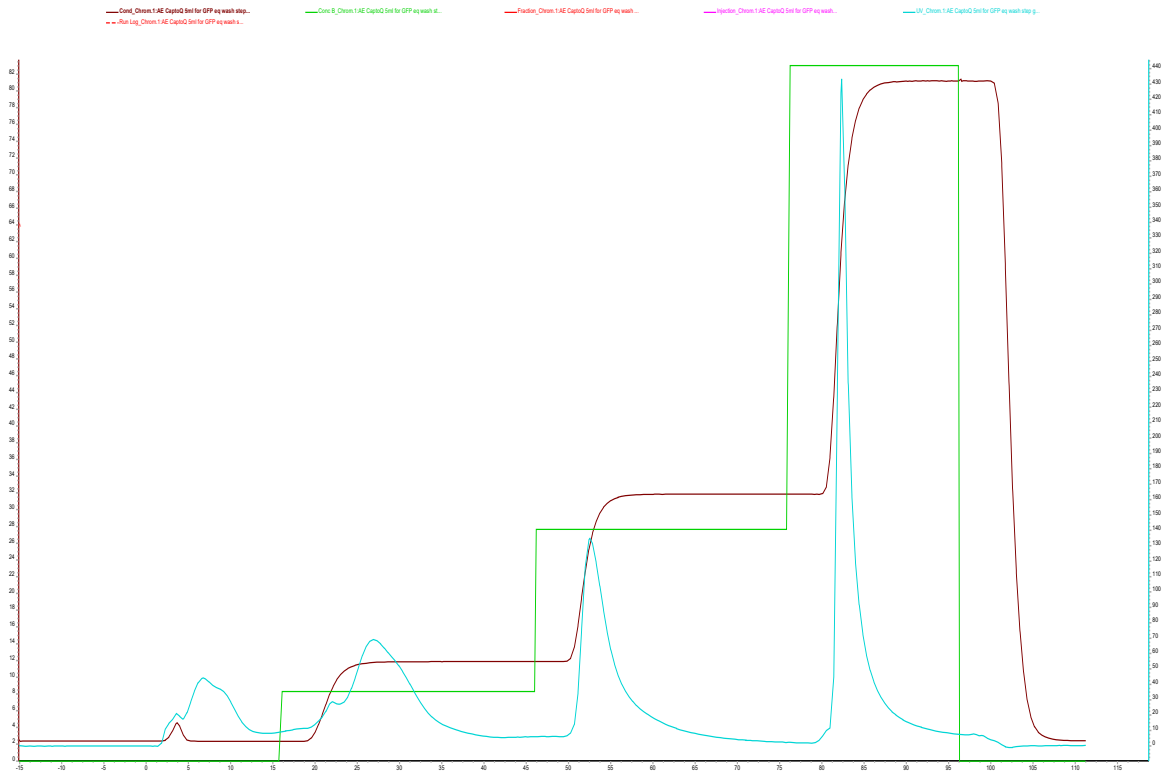
Flow rate 5.0 ml/min

Inlets A1 and B1, 50% A 50% B

Fill system 15ml

Wash 5 column volumes

Attachment 5. Anion Exchange of E. Coli cell lysate Chromatogram



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10. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	10MAR2019	Robin Zuck	Initial release