SOP Title:	**Standardization and Cleaning of the AKTA Pilot 600R Chromatography System**
SOP Number:	**14155**
Revision:	**02**

TABLE OF CONTENTS

1. PURPOSE 1
2. SCOPE 1
3. RESPONSIBILITIES 1
4. MATERIALS AND REAGENTS 2
5. STANDARDIZATION OF THE AKTA PILOT PH PROBE 2
6. CLEANING 3
7. STORAGE 7
8. REFERENCES AND RELATED DOCUMENTS 8

1. PURPOSE

This procedure describes the standardization of the pH monitor for the AKTA Pilot 600R Chromatography System, and the cleaning procedure for the system. SOP 14142 is also applicable for AKTA Pilot 600R standardization and cleaning.

2. SCOPE

This SOP applies to BDP personnel who perform standardization of the pH monitor and cleaning of the AKTA Pilot 600R Chromatography System. The AKTA Pilot 600R conductivity monitor calibration is part of AKTA Pilot 600R PM service performed by Cytiva Engineers.

3. RESPONSIBILITIES

3.1 Manager, Late Process Sciences, Biopharmaceutical Development Program (BDP)

- Defines the procedure.

3.2 BDP personnel

- Implements the procedure.

3.3 Biopharmaceutical Quality Assurance (BQA)

- Provides quality oversight.

4. MATERIALS AND REAGENTS

Part Number	Description	BDP Approved Substitution Permitted?
20315	Wipes, Sterile, 12x12	☒ YES ☐ NO
30129	70% Isopropyl Alcohol-Sterile, Decon-ahol, Sterile	☒ YES ☐ NO
46109	0.5 N NaOH	☒ YES ☐ NO
46202	20% Ethanol	☒ YES ☐ NO
20473	pH Strips	☒ YES ☐ NO
20006	15 mL polypropylene screw cap centrifuge tube	☒ YES ☐ NO
21875	T-connector, 5/16-24 AKTAPILOT	☒ YES ☐ NO
30107	pH 4 buffer	☒ YES ☐ NO
30108	pH 7 buffer	☒ YES ☐ NO
30109	pH 10 buffer	☒ YES ☐ NO
N/A	WFI Quality Water	☒ YES ☐ NO

5. STANDARDIZATION OF THE AKTA PILOT PH PROBE

5.1 Attach a new pH probe to the AKTA Pilot.

5.2 Turn on the AKTA Pilot and computer, if not already on.

5.3 Log into the Unicorn software.

5.4 Go to the system control menu > system > calibrate.

5.5 From the pull-down menu, select pH.

NOTE: Use the pH range as directed by the product run MPR else use the standard pH range for the calibration.

5.6 For the pH buffer 1, enter the pH value of first calibration solution.

NOTE: Use lowest pH buffer solution first to initiate the calibration.

5.7 Rinse the pH electrode with WFI and blot dry.

5.8 Place the pH electrode in the buffer 1 solution.

5.9 When the displayed current value is stable, press calibrate.

5.10 When the display changes to pH for buffer 2, enter the pH value of second calibration solution.

5.11 Rinse the pH electrode with WFI and blot dry.

5.12 Place the pH electrode in the pH buffer 2 solution.

5.13 When the displayed current value is stable, press calibrate.

5.14 Rinse the pH electrode with WFI and blot dry.

5.15 Document the slope and the asymmetry potential of the calibrationon Form 14155-03 and attach to applicable MPR.

6. CLEANING

6.1 Visually inspect the AKTA Chromatography System exterior and verify that it is clean. If necessary, use a cleanroom wipe and WFI followed by 70% IPA, wipe the exterior of the AKTA Pilot 600R Chromatography System to remove any visible contaminants.

6.2 Connect the AKTA Pilot 600R Chromatography System solvent lines (A1-A6 and B1-B3) to an appropriately sized container of ambient WFI.

NOTE: AKTA Pilot 600R can be bought with different configurations which may have a varying number of valves or sample pumps. The valves and pumps, listed in the previous step, are the maximum number the unit could potentially have installed.

6.3 Connect the Outlet valve lines (F2-F6), W1-W2 line, and bubble trap purge line in an appropriately sized waste container.

6.4 Evaluate what operations the unit was previously used for and proceed to the appropriate steps for any subsequent cleaning operations and complete Forms 12149-01 and 14155-02 as appropriate.

6.4.1 Not cleaned, previously used for non-viral product processing, proceed to step 6.5.

6.4.2 Not cleaned, previously used for viral product processing, proceed to step 6.11.

6.4.3 Cleaned and stored on 20% Ethanol, proceed to step 6.5.

6.4.4 Post maintenance work, proceed to step 6.5.

6.5 Ethanol Stored/Non-Viral Contact Activities

6.5.1 If the unit was previously stored on 20% Ethanol or was exposed to non-viral products or was subject to routine maintenance, use ambient WFI and flush each of the AKTA Pilot 600R Chromatography System Outlet and Waste lines (F2-F6 and W1- W2) through each of the solvent lines (A1-A6 and B1-B3) and column 1 and 2 positions. Start a manual run or use the AKTA Pilot 600R System Flush program and verify that each line is flushed at a minimum flow rate of 100 mL/min for at least 2 minute.

6.5.2 If a manual run is initiated, a suggested procedure is outlined below.

- Flush solvent line A1 to waste line W2
- Flush solvent line A2 to waste line W2
- Flush solvent line A3 to waste line W2
- Flush solvent line A1 to outlet line W1 with column 1 down
- Flush solvent line A1 to outlet line W1 with column 2 down
- Flush solvent line A2 to outlet line W1 with column 1 up
- Flush solvent line A3 to outlet line W1 with column 2 up
- Flush solvent line A1 to outlet line W1 with bubble trap online, column 1 bypassed, and column 2 bypassed.
- Flush solvent line A1 to outlet line F2 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line A2 to outlet line F3 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line A3 to outlet line F4 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line A1 to outlet line F5 with bubble trap offline, column 1 bypassed, and column 2 bypassed
- Flush solvent line A1 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed

- Flush solvent line A4 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line A5 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line A6 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line B1 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line B2 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed.
- Flush solvent line B3 to outlet line F6 with bubble trap offline, column 1 bypassed, and column 2 bypassed.

6.6 After completing the initial WFI flush, complete the appropriate section on Form 14155-02, connect an appropriately sized container of 0.5N NaOH.to the AKTA Pilot 600R Inlet valve lines.

6.7 Either manually or using the AKTA Pilot 600R System Flush program, flush each of the AKTA Pilot 600R Chromatography System Outlet and Waste lines (F1-F6 and W1-W2) with 0.5N NaOH through each of the solvent lines (A1-A6 and B1-B3) and column 1 and 2 positions.

6.8 Verify that the pH of the flowthrough from each of these lines is greater than or equal to 11 using pH strips. If the pH does not meet specification, continue flushing until the desired pH is achieved.

6.9 Upon meeting the required flowthrough pH, allow the AKTA Pilot 600R Chromatography System to be exposed to 0.5 N NaOH for ≥ 60 minutes.

6.10 After completion of the NaOH hold period, complete the NaOH exposure information on Form 14155-02 and proceed to step 6.12 for WFI flushing.

6.11 Viral Product Cleaning

6.11.1 If the unit was previously used for viral products and not cleaned, obtain an appropriately sized container of 0.5N NaOH and connect it to the AKTA Pilot 600R Chromatography System. Direct the system flowthrough(s) to an appropriate container for Potentially Infectious Material as required by **SOP 17109, Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious or Potentially Infectious Materials.**

6.11.2 Either manually or using the AKTA Pilot 600R System Flush program, flush each of the AKTA Pilot 600R Chromatography System Outlet and Waste lines (F1-F6 and W1-W2) with 0.5N NaOH through each of the solvent lines (A1-A6 and B1-B3) and column 1 and 2 positions.

The specific outlet and waste lines to be flushed should be referenced from the most recently executed MPR used prior to this cleaning operation. Not all of the outlet and waste lines listed above may need to be exposed to NaOH if they were not utilized during the purification of the last viral product purification.

6.11.3 Verify that the pH of the flowthrough from each of these lines is greater than or equal to 11, as measured via pH strips. If the pH does not meet specification, continue flushing until the desired pH is achieved.

6.11.4 Upon meeting the required flowthrough pH, allow the AKTA Pilot 600R Chromatography System to be exposed to 0.5N NaOH for ≥24 hours.

6.11.5 After completion of the NaOH hold period, complete the 0.5N NaOH exposure information on Form 14155-02 and proceed to step 6.12 for WFI flushing.

6.12 Post Cleaning WFI Flush

6.12.1 Upon completion of the 0.5N NaOH exposure hold, connect the AKTA Pilot 600R inlet valve to an appropriate container of ambient WFI.

6.12.2 Either manually or using the AKTA Pilot 600R System Flush program and using WFI (from step 6.12.1) flush each of the AKTA Pilot 600R Chromatography System Outlet and Waste lines exposed to 0.5N NaOH from step 6.5 or 6.11.

If WFI flushing from step 6.11 Viral Product Cleaning, all waste flowthrough should be collected and held in an appropriate container for Potentially Infectious Material as required by SOP 17109, Procedure for Safe Handling, Decontamination, and Spill Cleanup of Infections or Potentially Infectious Material until the processing equipment has been released from any viral products as directed by the area supervisor or associated viral testing protocols.

6.12.3 While flushing each flow path and appropriate inlet/outlet, document all WFI flushing on Form 14155-02.

6.12.4 Verify that the conductivity of the flowthrough from each of the lines is less than or equal to 1 mS/cm. If the conductivity does not meet the specification, continue flushing until the desired conductivity is achieved.

6.12.5 After properly flushing all appropriate inlet and waste/outlet ports, disconnect the tubing connections and take the testing samples as directed in the following steps.

6.12.6 To determine the appropriate post-cleaning sampling requirements, follow the guidance in steps 6.12.6.1 – 6.12.6.3. Use **SOP 22002, Request for Quality Control Testing**, for QC Sample Submission for any required samples that are to be submitted to BDP Quality Control/Process Analytics.

6.12.6.1 For In-Process cleaning: no testing is required unless directed by the MPR or area supervisor.

6.12.6.2 For Interbatch Cleaning / Post-Maintenance: collect a LAL rinse sample from W1, W2, F2, F3, F4 F5 and F6 lines. The LAL test specification is ≤ 0.5 EU/mL.

6.12.6.3 For Interproduct Cleaning: Follow the MPR and/or Sampling schedule and respective CTP (Interproduct Cleaning and Testing Protocol) for appropriate flow path or valve line selection and any required QC testing. **SOP 21529, Equipment Interproduct Cleaning and Clearance** should also be used in conjunction to collect all necessary cleaning samples.

6.12.7 Document all cleaning activities on Form 12149-01 and proceed to step 7 for equipment storage.

7. STORAGE

7.1 If storing the AKTA Pilot System less the 24 hours before use, the system may be stored using WFI.

7.1.1 Disconnect the Inlet valve tubing's from the WFI and seal the open end with end cap.

7.1.2 Seal the open end of the Outlet valves tubings by endcap or hemostat.

7.1.3 Label the equipment per **SOP 14150, Labeling of cGMP Purification Equipment for Cleaning Status.**

7.2 If storing for greater than 24 hours, connect the AKTA Pilot 600R Inlet valve lines to 20% Ethanol.

7.2.1 Either manually or using the AKTA Pilot 600R System Flush program, flush each of the AKTA Pilot 600R Chromatography System Outlet and Waste lines (F1-F6 and W1-W2) with 20% Ethanol through each of the sample lines (1-4) and solvent lines (A1-A4 and B1-B4) and column 1 and 2 positions. Verify that each line is flushed at a minimum flow rate of 100 mL/min for at least 2 minutes.

7.2.2 Disconnect the Inlet valve tubing's from the 20% Ethanol and seal the open end with end cap.

7.2.3 Seal the open end of the Outlet valves tubing's by endcap or hemostat.

7.2.4 Label the equipment per **SOP 14150, Labeling of cGMP Purification Equipment for Cleaning Status.**

7.3 Document all cleaning activities in the equipment logbook, Form 14155-01 and all storage activities on Form 14155-02.

7.4 At the start of each purification lot all pathways of the AKTA Pilot 600R Chromatography System will be flushed with WFI until the conductivity of the effluent from all pathways is ≤ 5 µS/cm. Document this step in the applicable purification MPR.

8. REFERENCES AND RELATED DOCUMENTS

Document Number	Title
12149	General Cleaning of Process Equipment
14150	Labeling of cGMP Purification Equipment for Cleaning Status
17109	Procedures for Safe Handling, Decontamination, and Spill Cleanup of Infectious or Potentially Infectious Materials
21529	Equipment Interproduct Cleaning and Clearance
22002	Request for Quality Control Testing, for QC sample submission
12149-01	Equipment Cleaning
14155-01	AKTA Pilot Chromatography System Use Log

Document Number	Title
14155-02	Flow-path Cleaning and Storage of the AKTA Pilot 600R Chromatography System
14155-03	Standardization of the AKTA Pilot pH Monitor