

Packing AxiChrom 50 and 70 columns with ÄKTA avant 150

1 Introduction

This document is an Addendum to AxiChrom 50-100 User Manual, 28-9331-08, and describes packing of AxiChrom 50 and 70 columns with ÄKTA™ avant 150.

2 Specifications

Compatible systems

AxiChrom 50 and 70 columns are compatible with the ÄKTA avant 150 system and integrated in UNICORN control software with a predefined method for Intelligent Packing.

Packing flow rates

The table below describes the packing flow rates of columns connected to ÄKTA avant.

Column	Packing flow rate at 30 cm/h	Packing flow rate at 60 cm/h
AxiChrom 50	8.8 ml/min	17.7 ml/min
AxiChrom 70	17.7 ml/min	35.4 ml/min



3 Connect AxiChrom columns to ÄKTA avant

Introduction

This chapter describes the basic configuration for connecting AxiChrom 50 and 70 columns to ÄKTA avant 150.

Connection to ÄKTA avant 150

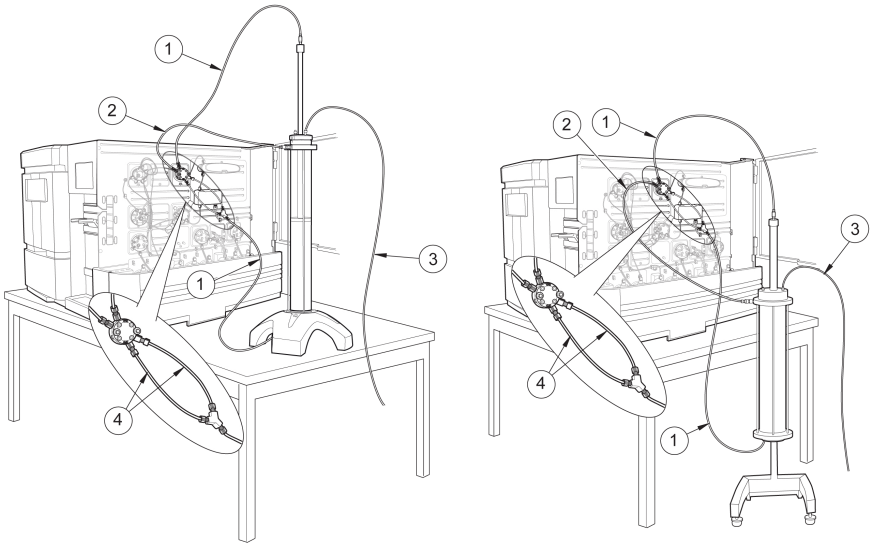
Connect the column to the system as described in the illustration in the *Intelligent Packing* phase and according to those column valves chosen in the method, see [Creating an Intelligent Packing method using GE approved media](#) step 6 on page 13.

Note: Use a T-connection on the outlet tubing.

Tubing used between column and system

Column diameter (mm)	ÄKTA system	Tubing i.d. (mm)
50	ÄKTA avant 150	1.7
70	ÄKTA avant 150	1.7

The illustration shows tubing connections for ÄKTA avant 150.



Pos.	28-9888-89 Tubing kit, AxiChrom 50 / ÄKTA avant / desk	28-9888-92 Tubing kit, AxiChrom 50-70 / ÄKTA avant / floor
1	id. 1.7 / L=1200 (x2)	id. 1.7 / L=1600 (x2)
2	id. 1.7 / L=800 (x1)	id. 1.7 / L=1200 (x1)
3	id. 2.9 / L=1600 (x1)	id. 2.9 / L=1600 (x1)
4	id. 1.7 / L=200 (x2)	id. 1.7 / L=200 (x2)

4 Create column type

Introduction

Before creating the Intelligent Packing method in UNICORN a column type needs to be created with the **Column Handling** tool. This type consists of:

- AxiChrom column hardware
- Medium
- Pressure/flow parameters
- Bed height

For more information on Column Handling, refer to the documentation for your UNICORN software.

Open the Column Handling dialog

To open the **Column Handling** dialog:

- select **Tools:Column Handling...** in any of the UNICORN modules
or
- click the **Column Handling** icon in the **Toolbar** where available

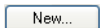


Create a new column type

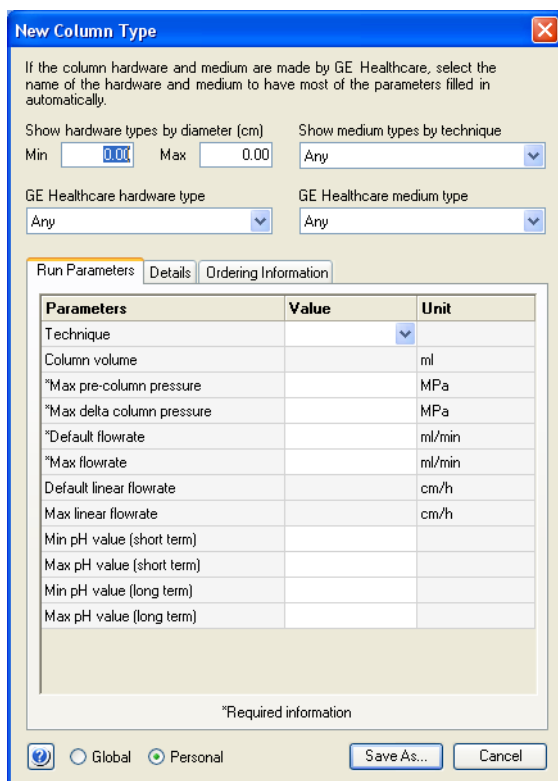
The table below describes how to add a new column type with the **Column Handling** tool:

Step Action

- 1 In the **Column Type Parameters** tab in the **Column Handling** dialog, click

.

Result: The **New Column Type** dialog opens.



The **New Column Type** dialog box is shown. It has a title bar with a close button. The main area contains instructions: "If the column hardware and medium are made by GE Healthcare, select the name of the hardware and medium to have most of the parameters filled in automatically." Below this are four dropdown menus: "Show hardware types by diameter (cm)" (Min: 0.00, Max: 0.00), "Show medium types by technique" (Any), "GE Healthcare hardware type" (Any), and "GE Healthcare medium type" (Any). There are three tabs: "Run Parameters" (selected), "Details", and "Ordering Information". The "Run Parameters" tab contains a table with columns "Parameters", "Value", and "Unit". The table lists various parameters and their units. At the bottom of the dialog are radio buttons for "Global" and "Personal" (selected), and "Save As..." and "Cancel" buttons.

Parameters	Value	Unit
Technique		
Column volume		ml
*Max pre-column pressure		MPa
*Max delta column pressure		MPa
*Default flowrate		ml/min
*Max flowrate		ml/min
Default linear flowrate		cm/h
Max linear flowrate		cm/h
Min pH value (short term)		
Max pH value (short term)		
Min pH value (long term)		
Max pH value (long term)		

*Required information

Note: The Intelligent Packing method is only compatible with AxiChrom columns.

2

- Select the appropriate AxiChrom hardware in the drop-down list **GE Healthcare hardware type** for the new column type.
- To filter the drop-down list to only show hardware types with certain diameters, enter the diameter range in cm in the **Min** and **Max** fields for **Show hardware types by diameter (cm)** above.
- Select the **GE Healthcare medium type** for the new column type in the drop-down list.
- To filter the drop-down list to only show medium types for a specific separation technique, choose the appropriate technique in the **Show medium types by technique** drop-down list above.

Result: The following parameters are automatically filled in (can be edited if appropriate):

Run Parameters		
Parameters	Value	Unit
*Technique	Anion Exchange	
Column volume		ml
*Max pre-column pressure		1.0 MPa
*Max delta column pressure		MPa
*Default flow rate		ml/min
*Max flow rate		ml/min
Default linear flow rate		cm/h
Max linear flow rate		cm/h
Min pH value (short term)		2
Max pH value (short term)		14
Min pH value (long term)		2
Max pH value (long term)		12

Details		
Parameters	Value	Unit
*Hardware diameter	5.0	cm
*Bed height		cm
Typical loading range		mg
Total liquid volume (V _l)		ml
Void volume (V ₀)		ml
Typical peak width at base		ml
Average particle diameter	90.0	
Molecular weight range		

Ordering Information	
Parameters	Value
Name	
Code number	
Medium name	Capto Q
Medium code number	17-5316-02
Hardware name	AxiChrom 50/300 glass, 20 um steel
Hardware code number	See user manual for AxiChrom

3

Enter the remaining parameter values for the new column type in the **Run Parameters**, **Details** and **Ordering Information** tabs. Fields marked with * must be filled in.

Values in the gray fields are calculated and automatically filled in based on entered values for the corresponding parameters.

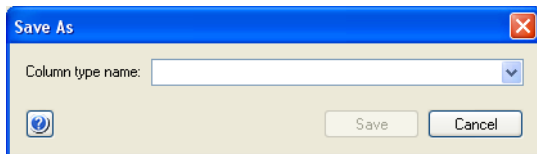
4

Select whether the new column type should be **Global** (available for all users) or **Personal** (only available for the current user).

Step	Action
------	--------

- | | |
|---|--|
| 5 | Click Save As... to save the column type. |
|---|--|

Result: The **Save As** dialog opens.



- | | |
|---|---|
| 6 | Type in a Column type name and click Save . |
|---|---|

Result: The column type is saved in the database and displayed in the **Column types** list.

Note: If traceability of result is requested, a column individual can be created under column type for logging of the data, see *UNICORN 6.1 Method Manual*.

5 Creating an Intelligent Packing method using ÄKTA avant 150

Introduction

This chapter contains some additional information to the chapter Intelligent Packing of the column in the *AxiChrom 50, 70 and 100 columns User Manual* where the Intelligent Packing wizard is presented for ÄKTApilot™ and ÄKTAexplorer™. This chapter describes how to create an Intelligent Packing method for ÄKTA avant 150 based on either GE approved media or custom media.

Creating an Intelligent Packing method using GE approved media

This section describes the creation of an example Intelligent Packing method based on GE approved media. If custom media is selected instead, the method settings allow a wider range of variables to be defined to optimize the method.

Follow the instruction below to create an Intelligent Packing method based on GE approved media.

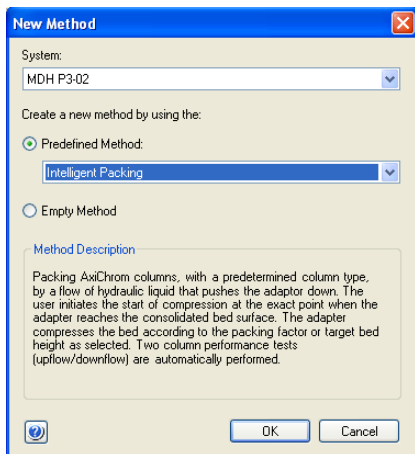
Step	Action
------	--------

- | | |
|---|---|
| 1 | Open the Method Editor module and click on the New method icon. |
|---|---|



Result: The **New Method** dialog opens.

- 2 Select **System** and **Predefined Method: Intelligent Packing** in the dialog. Click **OK**.



Result: The phases included in the chosen method are displayed in the **Method Outline** pane to the left, and the default settings for each of the phases are shown in the **Phase Properties** pane to the right.

3

In the **Phase Properties** pane of the **Method Settings** phase, edit general settings like **Column type** and **Method Base Unit**. UNICORN automatically calculates correct settings for volume, flow rate, and pressure limits based on the selected column type.

Note: Only AxiChrom columns can be selected in order to be able to run the method.

The illustration below shows the **Method Outline** pane to the left and the **Phase Properties** pane of the **Method Settings** phase to the right.

The screenshot displays the UNICORN software interface. On the left, the **Method Outline** pane contains a vertical stack of buttons: **Method Settings** (highlighted in blue), **Intelligent Packing**, **Equilibration**, **Column Performance Test**, and another **Column Performance Test**. The main window shows the **Phase Properties** pane for the **Method Settings** phase. This pane includes several configuration sections:
Column selection: Shows by technique (Intelligent Packing), Column type (AxiChrom 50/300 20 um Capto Q 20 cm), Column volume (392.639 ml), Pressure limit pre-column (1.00 MPa), Pressure limit delta-column (1.00 MPa), and a checked **Use flow restrictor** option.
Flow rate: Set to 458.3 cm/h with a range of [0.0 - 458.3] and a checked **Control the flow to avoid overpressure** option.
Buffer settings: Includes **Use manually prepared buffers** (selected) with Inlet A (A1) and Inlet B (B1), and **Use BufferPro (automatic buffer preparation)** with a selected recipe (Acetate 0.1M NaCl - pH 3.8-5.4, PD).
Monitor settings: Shows Wavelengths (190 - 700 nm) and UV 1 (280 nm).
Enable pH monitoring: Checked.
Column logging of: Checked, including **Column Performance Test** and **CIP**.

Make sure that all parameter values are correct.

Note: If the check box **Column Performance Test** is checked, automatic logging of the result will be saved in the column logbook if a column individual is chosen.

4

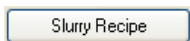
In the **Phase Properties** pane of the **Intelligent Packing** phase, make sure that the **GE approved packing settings** radio button is clicked. This button should be clicked if an AxiChrom column type with GE approved media was chosen in the Method Settings phase. Default parameter values for packing will automatically be filled in into the Intelligent Packing phase.

The illustration below shows the **Method Outline** pane to the left and the **Phase Properties** pane of the **Intelligent Packing** phase to the right.

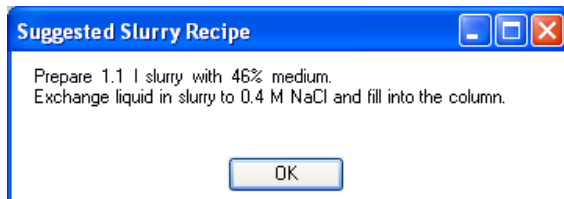
The screenshot displays two panes from a software interface. On the left is the 'Method Outline' pane, which contains a vertical stack of buttons: 'Method Settings', 'Intelligent Packing' (highlighted in blue), 'Equilibration', 'Column Performance Test', and 'Column Performance Test'. On the right is the 'Phase Properties' pane for the 'Intelligent Packing' phase. It features several settings: 'GE approved packing settings' is selected with a radio button, and 'Pack by Packing Factor' is selected with a radio button. The 'Pack Factor' is set to 1.15. Other settings include 'Adapter velocity' at 60.00 cm/h, 'Flow rate' at 0.0 cm/h, and 'Time' at 0.00 min. The 'Selected medium' is 'Capto Q', and the 'Target bed height' is 20 cm. The 'Slurry Concentration' is set to 40 %.

Note: If the user wants to make changes in parameter values, Custom packing settings can be used. See [Creating an Intelligent Packing method using custom media, on page 16](#).

- 5 In the **Phase Properties** pane of the **Intelligent Packing** phase, click on the **Slurry Recipe** button to receive a calculation of the amount of medium to fill in the column based on the slurry concentration. The recommended packing buffer for the selected medium is also shown in the dialog.



Result: The **Suggested Slurry Recipe** dialog opens.



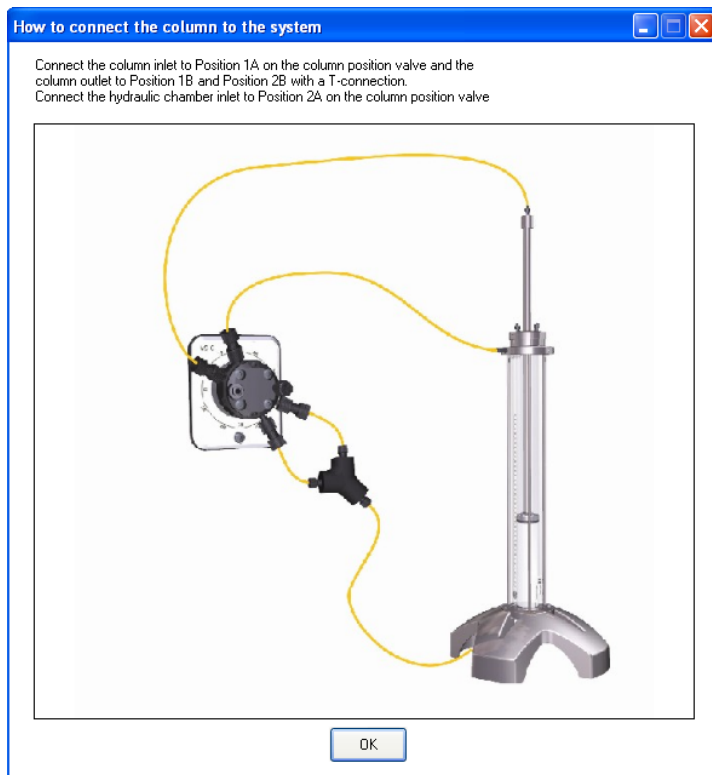
Note: The **Slurry Recipe** button is not available in Custom Packing settings.

Click **OK** to close the dialog.

- 6 In the **Phase Properties** pane of the **Intelligent Packing** phase, click on the **Column connection** button to display an illustration describing how to connect the column to the system.

Column Connection

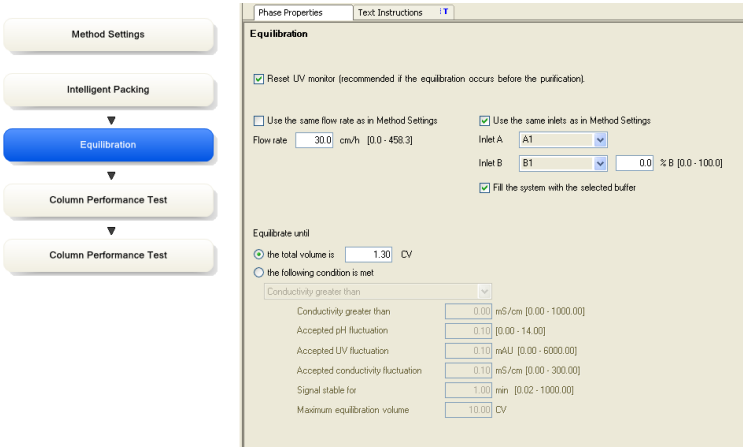
Result: The **How to connect the column to the system** dialog opens.



Note: The text will be updated dependent on the column positions chosen, but the illustration will remain the same.

Click **OK** to close the dialog.

- 7 In the **Phase Properties** pane of the **Equilibration** phase, make sure that the settings are correct. The default flow rate is set to 30 cm/h and the total volume of the Equilibration phase is 1.3 column volumes.
- The illustration below shows the **Method Outline** pane to the left and the **Phase Properties** pane of the **Equilibration** phase to the right.

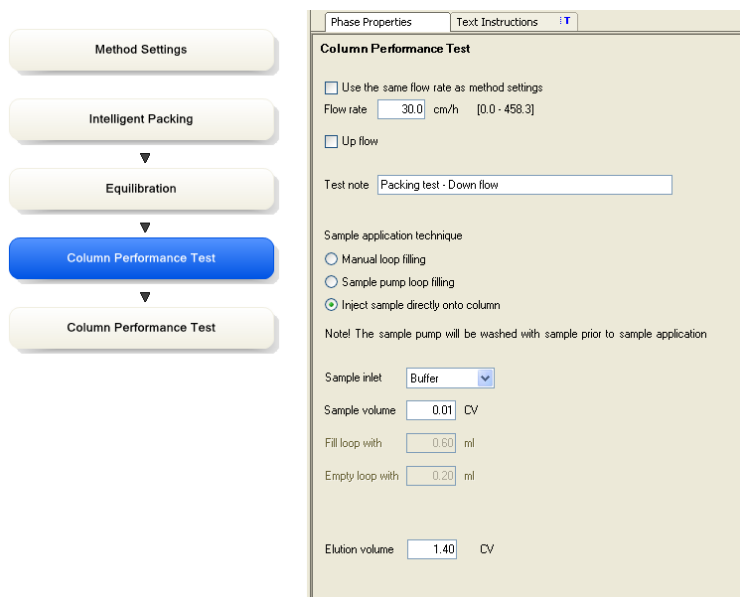


Note: All parameters in this phase can be changed.


- 8 In the **Phase Properties** pane of the **Column Performance Test** phase, make sure that the settings are correct. After the equilibration two Column Performance tests are run, one downflow and one upflow. Ensure that the **Upflow** check box is checked in one of the **Column Performance Test** phases. The test will then be run upflow. Ensure that the **Upflow** check box is unchecked in the other **Column Performance Test** phase and this test will be run downflow.

It is not necessary to run the tests, but is recommended to ensure the quality of the packed bed. A solution of either acetone or NaCl can be used to give a good indication of the column packing quality. The eluate is monitored by measuring the UV absorption at 280 nm or the conductivity and the curve can be evaluated in Evaluation. See *Performance evaluation of the column* in *AxiChrom 50, 70 and 100 columns User Manual* for more information.

The illustration below shows the **Method Outline** pane to the left and the **Phase Properties** pane of the **Column Performance Test** phase to the right.



Note: To check if the test is run upflow or downflow, see the **Upflow** check box. If this is checked, the test is run upflow.

Step	Action
	At both tests the flow rate is set to 30 cm/h. It is important to use a low flow and to use the same flow rate if tests are repeated to get comparable results.
9	Click the Save the method icon.
	
	<i>Result:</i> The Save As dialog opens.
10	In the Save As dialog: <ul style="list-style-type: none"> • Select a target folder to enable the Save button. • Type a Name for the method. • Select a System from the list. • Click the Save button.
	<i>Result:</i> The created method is saved in the selected folder.

Creating an Intelligent Packing method using custom media

Intelligent Packing methods may also be used for custom media. UNICORN facilitates this by providing an extended range of variables that may be modified to optimize the method. For example, it is possible to:

- Pack according to a desired Packing Factor or bed height. If selecting the latter option, the user stops the compression when the final bed height has been reached.
- Select flow conditioning with associated variables
- Specify desired adapter velocity during packing within defined range

In the **Phase Properties** pane of the **Intelligent Packing** phase, make sure that the **Custom packing settings** radio button is clicked.

The illustration below shows the **Method Outline** pane and the **Phase Properties** pane of the **Intelligent Packing** phase.

Method Settings

Intelligent Packing

▼

Equilibration

▼

Column Performance Test

▼

Column Performance Test

Phase Properties
Text Instructions
IT

Intelligent Packing

☐ GE approved packing settings

☒ Custom packing settings

GE Approved Media

Selected medium: Capto Q

Target bed height: 20 cm

Estimated time until bed contact warning: 30.4 min

Inlet for hydraulic chamber liquid

Inlet A A2 ▼

☒ Fill the system with the selected hydraulic chamber liquid

Column position for hydraulic chamber

Position 2 2 ▼

Column Connection

☒ Pack by Packing Factor

Pack Factor 1.20 [1.00 - 1.50]

☐ Pack to the target bed height

Adaptor velocity 50.00 cm/h [0.00 - 120.00]

☐ Flow conditioning

Flow rate 0.0 cm/h [0.0 - 458.3]

Time 0.00 min

Slurry Concentration (as measured in 20 % ethanol) 41 % [41 - 60]

Slurry Recipe

Inlet for mobile phase

☒ Use the same inlets as in Method Settings

Inlet A A1 ▼

Inlet B B1 ▼ 0.0 % B [0.0 - 100.0]

Make the appropriate changes and save the method in the same way as for GE approved packing.

6 Preparing the slurry, priming and packing the column using ÄKTA avant

Follow the instructions in the *AxiChrom 50, 70 and 100 columns User Manual* to prepare the slurry, prime the column, pour slurry into the column and to pack the column. The UNICORN pages may differ slightly for ÄKTA avant 150 but the concept is the same as for ÄKTAexplorer and ÄKTApilot.

For local office contact information,
visit

www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB

Björkgatan 30

751 84 Uppsala

Sweden

www.gelifesciences.com/axichrom

GE, imagination at work and GE monogram are trademarks of General Electric Company.

AxiChrom, ÄKTAexplorer, ÄKTApilot, ÄKTA and Drop Design are trademarks of GE Healthcare companies.

© 2010 General Electric Company – All rights reserved.
First published Dec. 2010

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available on request. Contact your local GE Healthcare representative for the most current information.

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327,
USA

GE Healthcare Japan Corporation
Sanken Bldg.3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan



imagination at work