

# Prepare buffers in plates and tubes with ÄKTA avant

## Background

Manual buffer preparation for high-throughput process development (HTPD) is complex and time consuming as the number of different buffer conditions can be large.

In this document you can find information on how to use the BufferPro function in ÄKTA avant to prepare buffers in plates and tubes as an alternative to a robotic system.

Using ÄKTA avant will significantly reduce manual work and add control with in-line pH and conductivity recordings during buffer preparation. However, buffer consumption will increase due to washing steps.

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## Plates and tubes

The BufferPro module and the fraction collector in ÄKTA avant can be used to fill plates and tubes with buffers.

The method can be adjusted to be used with the formats:

- Plates: 24, 48, 96 wells
- Tubes: 8, 15, 50, 250 ml

The salt concentration, which is set as %B, and pH can be varied in different wells or tubes.

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## Workflow

The workflow shows the different steps involved in using the ÄKTA avant BufferPro function for preparing buffers in plates or tubes. Each step in the workflow is detailed in the following sections.

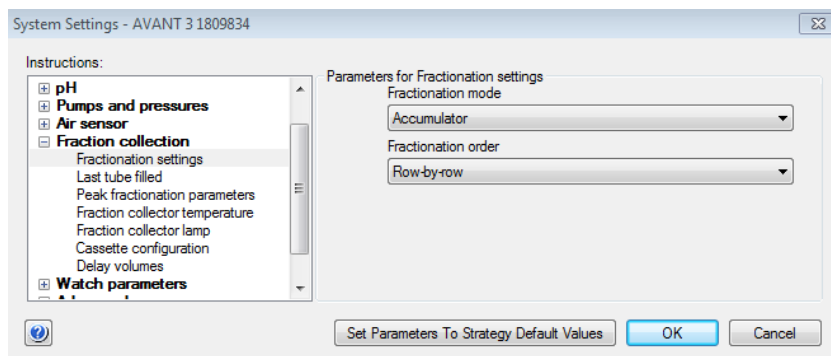
Stage	Description
1	Set fraction collection parameters in <b>System Control</b> in UNICORN™.
2	Import and open a method in <b>Method Editor</b> .
3	Select buffer system in <b>BufferPro</b> .
4	Enter settings for relevant plates or tubes.
5	Set pH and %B buffer (salt).
6	Prepare flow path and buffers.

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## 1. Set fraction collection parameters in System Control

- | Step | Action   |
|------|--|
| a    | Select <b>System:Settings</b> .  |
| b    | <ul style="list-style-type: none"><li>Expand <b>Fraction collection</b>.</li><li>Select <b>Fractionation settings</b> and set <b>Fractionation order</b> to <b>Row-by-row</b>.</li></ul> |



The image below illustrates the order of fractionation.

	1	2	3	4	5	6
A	1	2	3	4	5	6
B	7	8	9	10	11	12
C	13	14	15	16	17	18
D	19	20	21	22	23	24

## 2. Import and open a method in Method Editor

- | Step | Action  |
|------|---|
| a    | Import the method <code>BufferPro24well.editedAB</code> into the <b>Method Editor</b> . |
| b    | In the <b>Method Editor</b> , select <b>File:Open Method</b> .                          |
| c    | Select the method <code>BufferPro24well.editedAB</code> .                               |

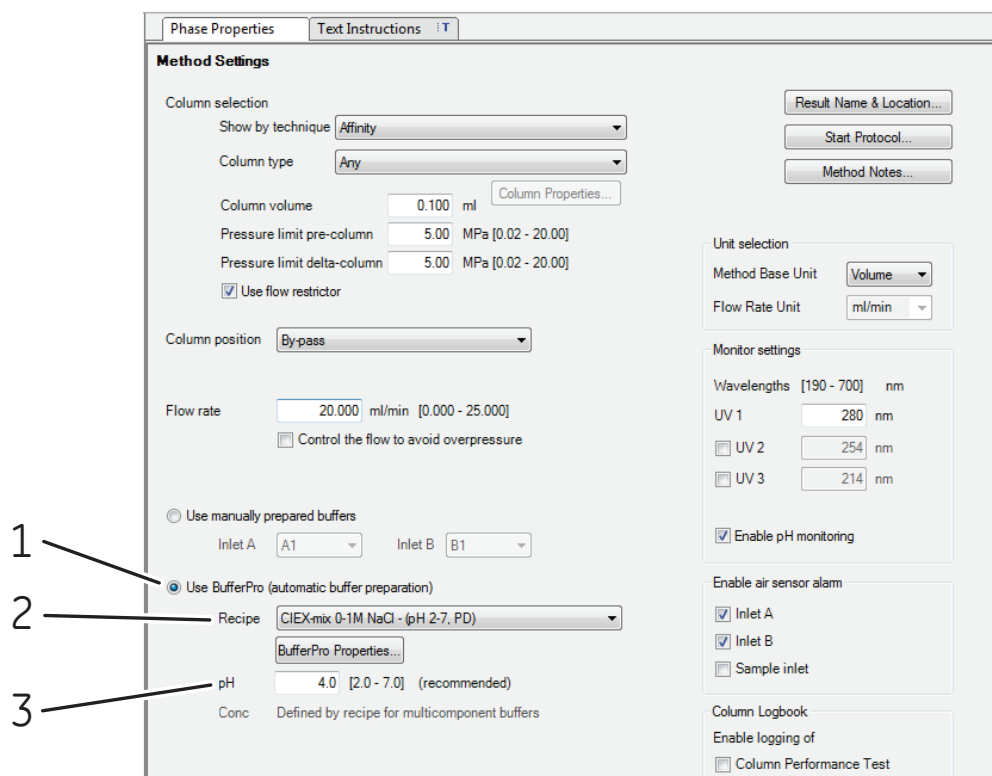
### 3. Select buffer system in *BufferPro*

Step      Action

a      Click the phase **Method Settings**.



b      Click the radio button **Use BufferPro (1)** and select buffer system from the **Recipe** drop down list (2).



**Note:**

*pH (3) is selected as scouting parameter in step 5.*

## 4. Enter settings for relevant plates or tubes

- | Step | Action   |
|------|--|
| a    | Click the phase <b>Prepare and fill selected pH and salt</b> . |

Prepare and fill selected pH and salt

- |   |  |
|---|--|
| b | Select plate or tube type (1): 24, 48 or 96 well plates, or 8, 15, 50 or 250 ml tubes. |
|---|--|

Phase Properties | Text Instructions

**Prepare and fill selected pH and salt** (This phase has been text-edited.)

Phase Variables

Block	Variable	Value	Range
PREPARE AND FILL SELECTED PH AND SALT	Percent B {%B}	0.0	[0.0 - 100.0]
Start frac (Elution)	Last tube filled action (Elution)	Pause	
	Frac tube type (Elution)	24 deep well p	
	Frac volume (Elution) {ml}	10.0	[0.0 - 10.0]
Single step gradient	Volume of well {ml}	9.50	[0.0 - 10.0]

- |   |  |
|---|--|
| c | Set <b>Frac volume</b> (2) to the maximum volume of the well or tube type. |
| d | Enter <b>Volume of well</b> (3).   |

**Note:**

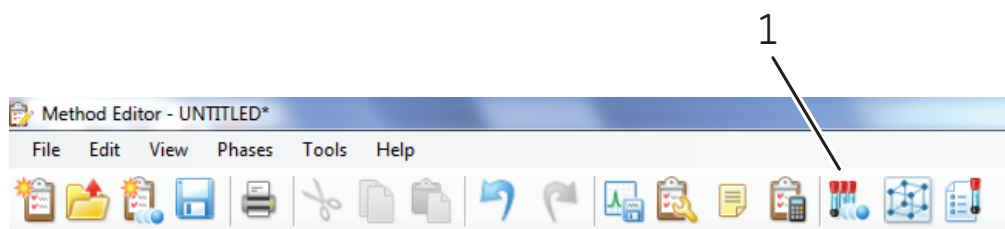
**Volume of well** should be set to 5% less than **Frac volume** to avoid spillage between wells or tubes.

The figure above shows an example of a method where a 24 deep well plate is used.

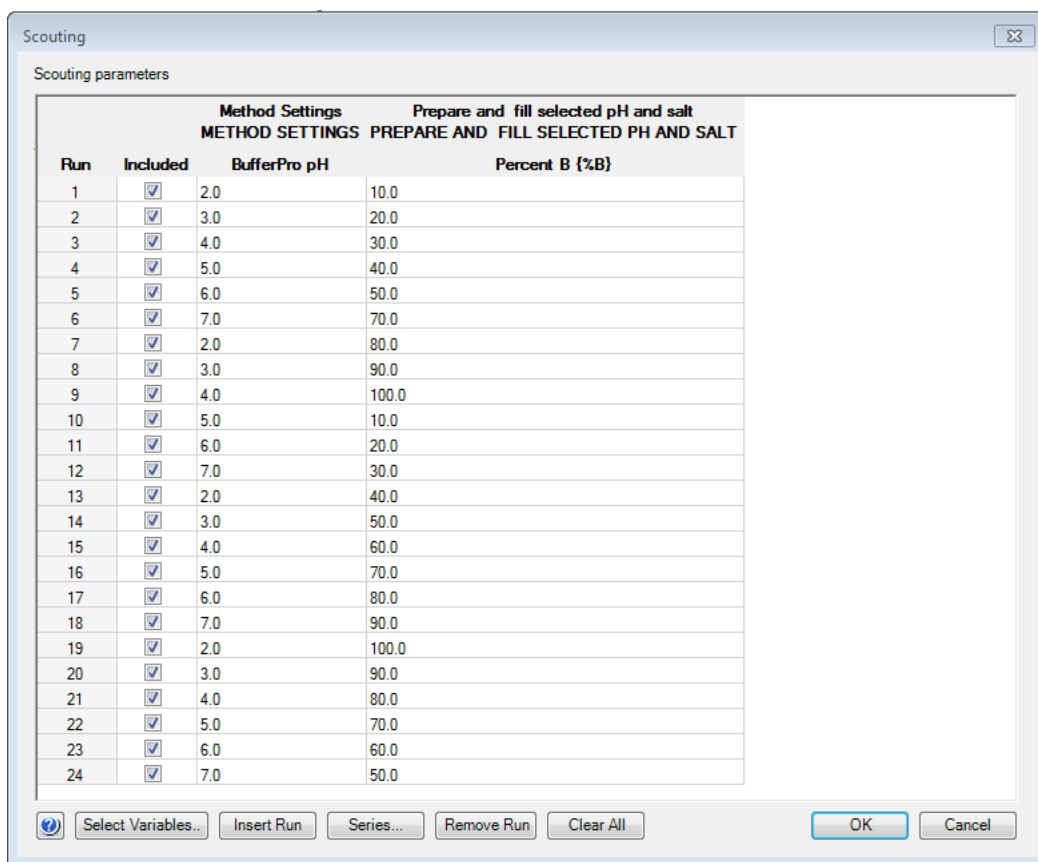
## 5. Set pH and % B buffer (salt)

Step Action

- a Click the Scouting icon (1) to open the **Scouting** dialog.



- b Set pH and %B buffer (salt) for each well or tube in the **Scouting parameters** dialog box.



**Note:**

100 %B corresponds to 1 M salt.

- c Click **OK**.

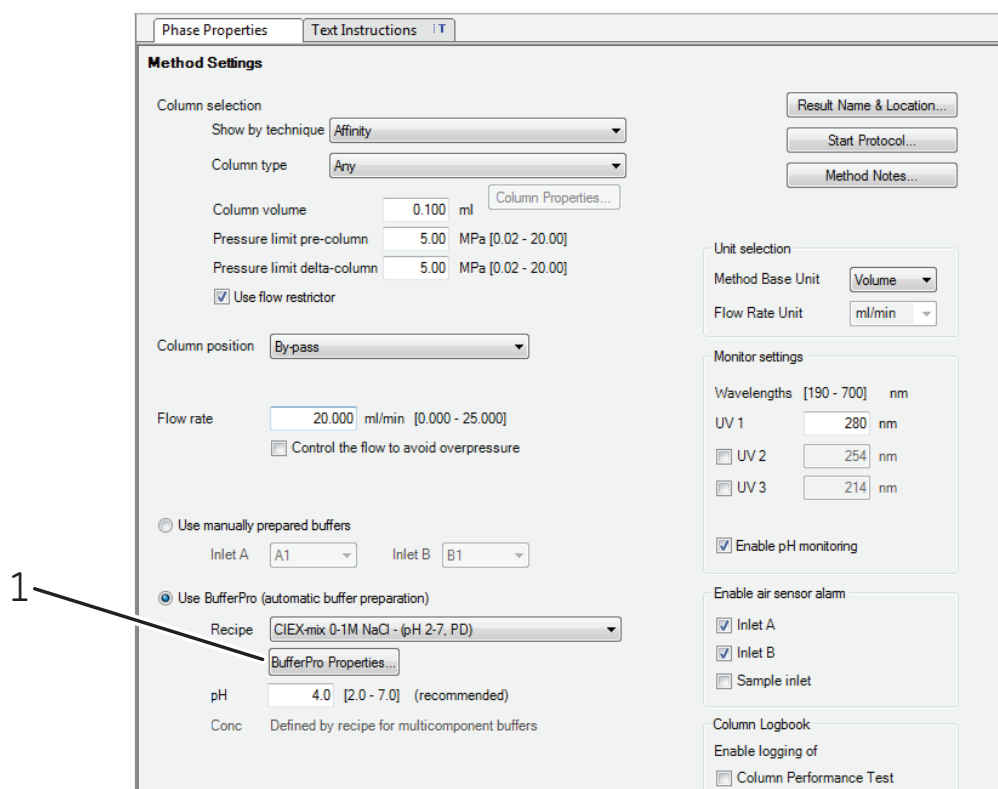
## 6. Prepare flow path and buffers

Step Action

a Click the phase **Method Settings**.



b Prepare tubing, stock solutions as advised in **BufferPro Properties (1)**.

A screenshot of the "Method Settings" dialog box in a software interface. The dialog has two tabs: "Phase Properties" and "Text Instructions". The "Method Settings" section includes:

- Column selection: "Show by technique" set to "Affinity", "Column type" set to "Any", "Column volume" set to "0.100 ml", "Pressure limit pre-column" set to "5.00 MPa [0.02 - 20.00]", "Pressure limit delta-column" set to "5.00 MPa [0.02 - 20.00]", and a checked "Use flow restrictor" checkbox.
- Column position: "By-pass".
- Flow rate: "20.000 ml/min [0.000 - 25.000]" with a checked "Control the flow to avoid overpressure" checkbox.
- Buffer settings: "Use manually prepared buffers" is unselected, and "Use BufferPro (automatic buffer preparation)" is selected. "Inlet A" is "A1" and "Inlet B" is "B1". The "Recipe" is "CIEX-mix 0-1M NaCl - (pH 2-7, PD)" and the "pH" is "4.0 [2.0 - 7.0] (recommended)". A "BufferPro Properties..." button is highlighted with a blue arrow and the number "1".
- Other settings: "Conc" is "Defined by recipe for multicomponent buffers".

On the right side, there are buttons for "Result Name & Location...", "Start Protocol...", and "Method Notes...". Below these are "Unit selection" (Method Base Unit: "Volume", Flow Rate Unit: "ml/min") and "Monitor settings" (Wavelengths: "190 - 700" nm, UV 1: "280" nm, UV 2: "254" nm, UV 3: "214" nm, and "Enable pH monitoring" checked). At the bottom right, there are "Enable air sensor alarm" (Inlet A and Inlet B checked, Sample inlet unchecked) and "Column Logbook" (Enable logging of: "Column Performance Test" unchecked).

**Note:** When performing scouting do NOT open the fraction collector door between runs. Opening the fraction collector door will reset the fraction collector and buffers will be dispensed into the wrong well or tube.

### Additional information

Additional information about the BufferPro function can be found in the ÄKTA avant user documentation as well as in the UNICORN user documentation.

Help texts for instructions can be accessed by selecting an instruction in the UNICORN **Instruction Box** or **Manual Instruction**, and pressing the **F1** key.

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