

Prepare buffers in plates and tubes with **ÄKTA** avant Instructions

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.

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.

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.

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

Set fraction collection parameters in **System Control**

Step	Action
1	Select System → Settings .
2	a. Expand <i>Fraction collection</i> .
	b. Select Fractionation settings and set Fractionation order to Row-by-row.

pH Pumps and pressures Air sensor Fraction collection Fraction settings Last tube filled Peak fractionation parameters Fraction collector temperature Fraction collector lamp Cassette configuration Delay volumes Watch parameters	-	Parameters for Fractionation settings Fractionation mode Accumulator Fractionation order Row-by-row
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The image below illustrates the order of fractionation.

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

Import and open a method in *Method Editor*

Step	Action
1	Import the method BufferPro24well.editedAB into the Method Editor.
2	In the <i>Method Editor</i> , select <i>File</i> → <i>Open Method</i> .
3	Select the method BufferPro24well.editedAB.

Select buffer system in **BufferPro**

Step	Action
1	Click the phase <i>Method Settings</i> .
	Method Settings
2	Click the radio button Use BufferPro (1) and select buffer system from the Recipe drop

down list (2).
Phase Properties Text Instructions IT

Method Settings				
Column selection				Result Name & Location.
Show by te	echnique Affinity	•		Start Protocol
Column ty	pe Any	•		Method Notes
Column ve		Column Properties		
		a [0.02 - 20.00]	Unit selection	
		a [0.02 - 20.00]	Method Base	Unit Volume 👻
Use flo	w restrictor		Flow Rate Uni	t ml/min 👻
Column position (By-pass	•	Monitor setting	s
			Wavelengths	[190 - 700] nm
Flow rate	20.000 ml/min [0.000 - 25.		UV 1	280 nm
	Control the flow to avoid overpr	essure	🔲 UV 2	254 nm
			🔲 UV 3	214 nm
Use manually pre	pared buffers			
Inlet A	A1 v Inlet B B1	-	📝 Enable pH	monitoring
Use BufferPro (a)	utomatic buffer preparation)		Enable air sens	sor alarm
Recipe	CIEX-mix 0-1M NaCl - (pH 2-7, PD)	•	✓ Inlet A	
	BufferPro Properties		Inlet B	
pH	4.0 [2.0 - 7.0] (recommer	nded)	Sample in	let
Conc I	Defined by recipe for multicompone	ent buffers	Column Logboo	ok
			Enable logging	g of
			Column Pe	erformance Test

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

Enter settings for relevant plates or tubes

Step	Action
1	Click the phase Prepare and fill selected pH and salt .
	Prepare and fill selected pH and salt : T

2

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

Phase Properties Text Instructions				
Trepare and fill selected pH and salt iT This phase has been text-edited.)				
Phase Variables Biock	Variable	Value	Range	
PREPARE AND FILL SELECTED PH AND SAL	F Percent B {%B}	0.0	[0.00.0]	
Start frac (Elution)	Last tube filled action (Elution)	Pause		-
	Frac tube type (Elution)	24 deep well p		
	Frac volume (Elution) {ml}	10.0	[U.0 - 10.0]	
Single step gradient	Volume of well {ml}	9.50 -		

3 Set *Frac volume* (2) to the maximum volume of the well or tube type.

4 Enter Volume of well (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.

Set pH and % B buffer (salt)

Step	Action
1	Click the Scouting icon (1) to open the Scouting dialog.
	1
	$\overline{\setminus}$
	By Method Editor - UNTITLED*
	File Edit View Phases Tools Help
	19 🖆 🚉 🖬 🖨 💊 🗈 👘 🥍 🥐 (* 🖳 🚉 🗉 💼

2

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

		Method Settings METHOD SETTINGS	Prepare and fill selected pH and salt PREPARE AND FILL SELECTED PH AND SALT	
Run	Included	BufferPro pH	Percent B {%B}	
1	V	2.0	10.0	
2	V	3.0	20.0	
3	V	4.0	30.0	
4	V	5.0	40.0	
5	V	6.0	50.0	
6	V	7.0	70.0	
7	V	2.0	80.0	
8	V	3.0	90.0	
9	V	4.0	100.0	
10	V	5.0	10.0	
11	V	6.0	20.0	
12	V	7.0	30.0	
13	V	2.0	40.0	
14	V	3.0	50.0	
15	V	4.0	60.0	
16	V	5.0	70.0	
17	V	6.0	80.0	
18	V	7.0	90.0	
19	V	2.0	100.0	
20	V	3.0	90.0	
21	V	4.0	80.0	
22	V	5.0	70.0	
23	V	6.0	60.0	
24	V	7.0	50.0	

Note:

100 %B corresponds to 1 M salt.

3 Click OK.

Prepare flow path and buffers

1-

Step	Action		
1		ase <i>Method Settings</i> . Nethod Settings	
2		ng, stock solutions as advised in BufferPro Proj	perties (1).
		Phase Properties Text Instructions IT Method Settings Column selection Show by technique Affinity Image: Column type Column type Arry Image: Column Properties Column volume 0.100 ml Pressure limit pre-column 5.00 MPa [0.02 - 20.00] Pressure limit delta-column 5.00 MPa [0.02 - 20.00] Image: Use flow restrictor Image: Column type Image: Column type	Result Name & Location Start Protocol Method Notes Unit selection Method Base Unit Volume Flow Rate Unit
		Column position By pass Flow rate 20.000 ml/min [0.000 - 25.000] Control the flow to avoid overpressure Use manually prepared buffers	Monitor settings Wavelengths [190 - 700] nm UV 1 280 nm UV 2 254 nm UV 3 214 nm

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.

Inlet A A1 - Inlet B B1

Recipe CIEX-mix 0-1M NaCl - (pH 2-7, PD)

Conc Defined by recipe for multicomponent buffers

4.0 [2.0 - 7.0] (recommended)

Use BufferPro (automatic buffer preparation)

pН

BufferPro Properties...

Enable pH monitoring

Enable air sensor alarm

🔽 Inlet A

🗸 Inlet B

Sample inlet

Column Logbook Enable logging of Column Performance Test

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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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