1. 硬件检查

- 1) USB 连接线是否连接好
- 2) 电源线是否插入电源插座中
- 3) 废液管是否在废液缸中

2. 开机

1) 先打开 ÄKTApure 电源,当控制面板上的 Power 灯稳定不再闪烁时打 开电脑,双击电脑桌面上的 UNICORN 6 图标打开软件

2) 选择用户,输入密码(如果进行了设置),点击 OK 进入软件

利 Log On - I	UNICORN
Use <u>W</u> ind	ows Authentication
<u>U</u> ser Name:	Default 🗸
<u>D</u> omain:	×
Access Group:	Administrators 💌
0	<u>OK</u> <u>Cancel</u> Options >>

3) 在任务栏中将出现四个软件窗口,单击系统控制 (System Control)

🦺 start 🔞 🧕 🖉 🎽 Administration 🙀 System Control 🎯 Method Editor 🔤 Evaluation

如果 System Control 窗口没有自动弹出,可在任意打开的窗口中点击 Tools > System Control 打开



4) 进入 System Control 窗口,点击 Connect to Systems,在弹出的对话 框中选中已连接的系统名称,点击 OK 确认连接

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Ľ						V 🗐	-				
<u> 1</u> Method Navigator									onnect to Systems		
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									Uonnected Users		Cancel

3. 实验前的准备

3.1 缓冲液准备

所有层析用的缓冲液及样品都需要用 0.22 μm 的滤膜过滤,并对缓冲液 进行脱气处理(如超声波脱气或者负压脱气)

3.2 泵头抽气

如果缓冲液进口管是空的或者有太多气泡,此时需要手动排气。在泵头 上方的抽气螺母上连接一个注射器,拧松螺母并抽气。泵头内的气泡也是导 致压力和流速不稳定的主要原因,也需要采用这种方式来排除泵头内的气泡



- 3.3 泵冲洗
 - 将进口管转移到缓冲液中:将缓冲液进口管从 20%乙醇保护液中转 移到相应的缓冲液瓶中。如果缓冲液含高盐,建议先将进口管转移到 去离子水中进行泵冲洗,然后再转移到相应缓冲液瓶中再进行泵冲洗



2) 在 System Control 界面的 Manual 下拉菜单中选择 Execute Manual Instructions



3) 在跳出的 Manual instructions 窗口中,选择 Pumps > Pump A wash, 选择需要冲洗的缓冲液入口,点击 Execute,进行泵的自动冲洗

Manual instructions - akta pure		×
Instructions: Pumps System flow Gradient Pump A wash Pump B wash Mixet by pass wash System wash Flow path Monitors Fraction collection Alams Wash settings	Selected column type: Select	
Save result as:	Browse Execute Close	

3.4 安装柱子

1) 在 Flow path 命令组中选择 Column position, 在 Position 下拉框中选择柱子需要连接的位置, 在 Flow Direction 复选框中选择溶液流向, 点击 Insert

Manual instructions - akta pure		
Instructions: Pumps Flow path Inlet A Inlet B Injection valve Column position pH valve Outlet valve Mixer valve Injection mark Mixer valve Injection mark Fraction collection	Selected column type: Select Instruction execution list: Parameters for Column position 2 Flow Direction O Down flow Up flow	
Save result as: Image: Save result as: Imag	Ing run	e Close

2) 在 Pumps 命令组中选择 System flow, 在 Flow rate 一栏中输入一个较 小的流速如 0.5-1 ml/min, 点击 Insert

Manual instructions - akta pure		
Instructions: System flow Gradient Pump A wash Pump B wash Mixer by-pass wash System wash E flow path Monitors Fraction collection Alarms Wash settings	Selected column type: Select Parameters for System flow Flow rate 0.55 ml/min Linear Flow Pressure control Off)
Save result as: Image: Save result as: Imag	Browse	Execute Close

3) 在 Alarms 命令组中选择 Alarm pre column pressure, 根据所使用柱子 的耐受压设置 High alarm, 如 0.3 MPa, 点击 Insert。 点击 Execute 后将执行插入的所有命令

Manual instructions - akta pure		
Instructions: Flow path Monitors Fraction collection Alarms Alarm system pressure Alarm pre column pressure Alarm pre column pressure Alarm DV1 Alarm conductivity Alarm pH Alarm inlet A air sensor Alarm inlet B air sensor	Selected column type: Select Instruction execution list: Mode O Disabled Instruction execution list: Mode O Disabled O Disabled High alarm (0.02 · 20.00) Column pressure Enabled, 0.30, 0.00 Low alarm (0.00 · 20.00) MPa Low alarm (0.00 • 20.00) Instruction execution list:	
Save result as:	Browse	
Auto update of parameters duri	ng run <u>Execute</u>	Close

4) 在柱位阀的相应位置上连接一根 PEEK 管,待 PEEK 管的出口有持续 液体流出时,除去层析柱的上堵头,将层析柱柱头与连接管出口相连, 但不要拧紧,因为此时出口尚未打开



5) 除去层析柱下堵头,并将层析柱出口连接到柱位阀的相应位置上,然 后拧紧上接头



6) 点击 end,完成准备工作

b	System C	ontrol						
Fi	le Edit	View	Manual	System	Tools	Help		
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1	i 🕘 akt	a pure						
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od N	System state Acc. volume System flow							D₩
lavi	Manual Ru	ın	0.1	151 ml		1.0	100 ml/m	in
gator								

- 4. 采用手动命令进行层析实验操作
- 4.1 平衡
 - 1) 检查缓冲液入口是否在合适的溶液中,在 Alarm pre column pressure 命令输入框中输入高压报警值,譬如 0.3 MPa,点击 Insert 插入命令

Manual instructions - akta pure		K
Instructions: Flow path Monitors Fraction collection Alarm system pressure Alarm delta column pressure Alarm UV1 Alarm pre column pressure Alarm pre column pressure Alarm pre column pressure Alarm pre column statistication of the s	Selected column type: <u>Select</u> Parameters for Alarm pre column pressure Mode Disabled © Enabled High alarm (0.02 - 20.00) Column (0.00 - 20.00) MPa Low alarm (0.00 - 20.00) 0.00 © MPa	
Save result as:	Browse Execute Close)

2) 在 Pumps 命令组中的 System flow 命令的输入框中输入层析柱的平衡 流速,点击 Insert

Manual instructions - akta pure	
Instructions: Pumps System flow Gradient Pump A wash Pump B wash Mixer by-pass wash System wash Flow path Monitors Fraction collection Alarms Wash settings	Selected column type: Select Parameters for System flow Flow rate 2 ml/min Linear Flow Pressure control Off
Save result as:	Browse Execute Close

3) 在 Flow path 命令组中的 Column position 下选择层析柱位置及液流方向,点击 Insert 插入命令

Manual instructions - akta pure			
Instructions: Pumps Flow path Inlet A Inlet B Injection valve Column position pH valve Outlet valve Mixer valve Injection mark Monitors Fraction collection	Selected column type: Parameters for Column position Position 2 Flow Direction ① Down flow ① Up flow	Select	Instruction execution list: Insert Delete Alarm pre column pressure Enabled, 0.30, 0.00 System flow 2.000, Dff Column position 2, Down flow
Save result as:	un	<u>B</u> rowse	Execute Close

4) 如果检测器默认的三个检测波长不是自己需要的,可进入 Monitors
 命令组在 Wavelength 命令的输入框中输入您需要的波长,并且可以
 通过选定 UV2 和 UV3 后面的 Off 复选框来关闭这两个波长,点击
 Insert 插入命令

Manual instructions - akta pure							×
Instructions: Pumps Flow path Monitors Auto zero UV Wavelength Noise reduction UV Relative scale cond Fraction collection Alarms Wash settings Watch parameters Advanced		Selected column type: Parameters for Wavelength UV 1 UV 2 UV 2 UV 3	[190 - 700] 280 = nm [190 - 700] 260 = nm [190 - 700] 0 = nm	☐ Off ✔ Off	Select	Instruction execution list: Insert Delete Alarm pre column pressure Enabled, 0.30, 0.00 System flow 2.000, 0.ff Column position 2, Down flow Wavelength 280, 250, 0ff	
Save result as:	ng run				<u>B</u> rowse	Execute Close	

5) 点击 Execute 执行所有插入的命令,系统开始对层析柱进行平衡,一般平衡 5-10 个柱床体积



6) 在层析柱平衡完成时,上样前通常还会做一个紫外调零的动作。在 Monitors 命令组中选择 Auto zero UV,点击 Execute 执行

Manual instructions - akta pure		
Instructions: Pumps Flow path Monitors Auto zero UV Wavelength Noise reduction UV Relative scale cond Fraction collection Alarms Wash settings Watch parameters Advanced	Selected column type: Select	Instruction execution list: Insert Delete Auto zero UV
Save result as: ∭ ▲uto update of parameters during rur	<u>B</u> rowse	Execute Close

- 4.2 上样
 - 1) 将合适的样品环连接于上样阀的 LoopF 与 LoopE 口上,用注射器抽 取平衡缓冲液,从 Syr 上样口推入,冲洗样品环



2) 用注射器吸取稍大于样品环体积的样品,将注射器内的样品从 Syr 口 推入,注意不要将气泡推入样品环



3) 在 Flow path 命令组中的 Injection valve 中选择 Inject,按 Execute 执行

Manual instructions - akta pure	δ	
Instructions: Pumps Flow path Indet A Indet A Indet B Injection valve Column position pH valve Outlet valve Mixer valve Injection mark Fraction collection	Selected column type: Select Parameters for Injection valve Position Inject Manual load Direct mject System pump waste Direct mject Sample pump load Sample pump waste	
Save result as:	Browse Execute Close	

4) 待上样完成后(完全上样通常需 2 倍以上样品环容积的缓冲液流过), 将上样阀状态切换为 Manual load,点击 Execute 执行,完成上样

Manual instructions - akta pure		
Instructions: Pumps Flow path Inlet A Inlet B Injection valve Column position pH valve Outlet valve Mixer valve Injection mark Monitors Fraction collection	Selected column type: Select	Instruction execution list: Insert Delete
Save result as:	ng run	Execute Close

5) 上样完成后继续使用平衡缓冲液冲洗 1~2 个柱体积,以洗掉不结合的物质(如果是凝胶过滤层析,忽略此步)

4.3 洗脱

对于吸附性层析往往需要改变洗脱液的洗脱强度来实现分离,这就需要 在洗脱时 A、B 泵相互配合来实现。在 Pumps 命令组中的 Gradient 中输入 目标洗脱缓冲液的比例,如果是阶段梯度洗脱在 Length 中输入 0 min, B 的 比例立即达到设定值;如果需要线性梯度洗脱,在 Length 中输入时间,洗 脱液将会在设定的时间内逐渐达到设定的比例

Manual instructions - akta pure		
Instructions: System flow Gradient Pump A wash Pump B wash Mixer by-pass wash System wash Flow path Monitors Fraction collection Alarms Wash settings	Selected column type: Select Instruction ex Target 20 2 %8 Length [0.00 - 100000.0] 0.000 min	ecution list: Delete D, 0.00
Save result as: Output Output Output Output	Browse	Execute Close

4.4 收集

1) 在 Flow path 命令组中将出口阀 Outlet valve 的位置由 Waste 改为 Frac

Manual instructions - akta pure			
Instructions: Pumps Pumps Inde tA Inde tA Inde tB Injection valve Column party valve Outlet valve Mixer valve Injection mark Monitors Praction collection V	Selected column type: Parameters for Outlet valve Position Frac	Select	Instruction execution list: Insert Delete Dutlet valve Frac
Save result as:	n	<u>B</u> rowse	Execute Close

2) 在 Fraction collection 命令组中的手动收集命令 Fractionation 中设置 每管收集的体积(或时间)Fraction size,点击 Execute 执行

Manual instructions - akta pure		
Instructions: Fraction collection Fractionation Stop fractionation Peak fractionation Reset frac number Fractionation in outlet valve Stop frac in outlet valve Stop frac in outlet valve Peak fractionation in outlet valve Peak fractionation parameters	Selected column type: Select	Instruction execution list: Insert Delete Outlet valve Frac Fractionation Volume, 1.00
Save result as:	growse	Execute Close

当收集的样品还没有达到设定的体积,而需要换到下一管时,可以执 行 Feed tube 命令进行跳管

Manual instructions - akta pure		<
Instructions: Fraction collection Fractionation Stop fractionation Peak fractionation Stop peak fractionation Reset frac number Fractionation in outlet valve Stop frac in outlet valve Peak frac in outlet valve	Selected column type: Parameters for Feed tube Parameters for Feed tube Feed tube Feed tube	
Save result as:	Browse	

3) 当需要停止收集时,执行 Stop fractionation 命令或者在 Fraction size 中输入 0,点击 Execute 执行

5. 层析柱的再生与保存

- 1) 在工具栏中点击 Pause,让系统暂停,然后将 B 泵缓冲液进口管 转移到再生缓冲液中(如 2M NaCl、1M NaOH 等),再点击 "Continue"继续运行一段时间
- 2) 在工具栏中点击 Pause,让系统暂停,然后将所有用过的缓冲液 进口管转移到纯水中,按 Continue,然后执行 PumpWash 命令, 将系统中更换成水,继续运行一段时间冲出柱子内的盐类等物质
- 3) 在工具栏中点击 Pause,让系统暂停,然后将所有用过的缓冲液 进口管转移到 20%乙醇中,按 Continue,然后执行 PumpWash 命 令,将系统中更换成 20%乙醇,继续运行一段时间将柱子内的液 体更换成 20%乙醇
- 4) 点击工具栏中的 End 按钮,结束层析试验
- 6. 结果处理与分析
- 6.1 打开结果
 - 1) 点击任务栏中的 Evaluation,进入结果处理窗口

```
🚀 Administration 🛛 🗛 Evaluation - [UNT... 🍺 Method Editor 🛛 🦓 System Control
```

如果任务栏中没有 Evaluation 窗口,可以在任意已打开的 UNICORN 6 窗口中点击 Tools,选择 Evaluation 打开



2) 在 Evaluation 窗口中点击 Open Results Navigator 打开 Results 文件导航窗口

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		De	faultHo	me				2/4/201	39
	-		akta p	ure (Manu	al)			2/4/201	31
			🔍 Ma	anual Run	001	akta p	oure	2/4/201	31
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			🛺 Ma	anual Run	004	akta p	oure	2/17/20	13
			🛄 Ma	inual Run	005	akta p	oure	2/17/20	13
			🛄 Ma	anual Run	006	akta p	oure	2/18/20	13
			🛄 Ma	anual Run	007	akta p	oure	2/18/20	13

双击待分析的结果文件打开



也可以在 Evaluation 窗口中点击 File > Open > Chromatograms 打开结 果选择窗口



在弹出的窗口中双击待分析的结果,勾选 Chrom 1,点击向右双箭头 选中结果后按 OK 键打开

Open Chromatograms				Σ
esuits, Folders	•			
Folder name	System	Last modified	Created by	Path 🗹
😑 🛛 📴 akta pure (Manual)		2/4/2013 11:14:5	Default	/Defa
🔍 Manual Run 001	akta pure	2/4/2013 11:16:5	Default	/
風 Manual Run 002	akta pure	2/6/2013 12:04:5	Default	1
風 Manual Run 003	akta pure	2/6/2013 12:07:2	Default	1
風 Manual Run 004	akta pure	2/17/2013 10:32:	Default	/
風 Manual Run 005	akta pure	2/17/2013 10:37:	Default	1
Anual Run 006	akta pure	2/18/2013 2:22:4	Default	1
🛛 🛄 Manual Run 007	akta pure	2/18/2013 11:39:	Default	/
				>
♥ Chrom 1 : Manual Run 006	→ 			
@		[OK	Cancel

还可以通过单击"最近的运行(Recent Runs)"选项,找到需要分析的层 析结果双击打开

۸	Evaluati	on -	[Manua	l Run 00	5*]				
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6.2 层析结果的显示设置

1) 正常打开的结果如下



2) 在 Curve 窗格点击鼠标右键,点击菜单中的 Customize



在复选框中选中需要显示的色谱曲线



点击 OK 即可使图谱简化,或者显示更丰富的曲线



6.3 结果积分处理

1) 在工具栏中点击峰积分按钮,进行积分处理

🛵 Evaluation - [Manual Run 005*]										
۵	Eile	<u>E</u> dit	⊻iew	Integrate	Operations	Procedures	<u>T</u> ools	Help		
	7 7	1 🖻	} 🔒		6 [19	٨			

或者在 Integrate 下拉菜单中点击 Peak Intergrate 进行积分处理

👆 Evaluation - [Manual Run 005*]								
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		⊆alcul	ate Baseline	Ctrl+B				
Chrom.1		<u>E</u> dit Ba	aseline	Ctrl+E				
Resul		Edit P	eak <u>T</u> able					

2) 选择需要积分的曲线以及积分表存储位置,基线类型默认为 Calculate baseline

Peak Integrate		X
Chromatogram: Chrom.1	∐arget peak table:	
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<u>B</u> aseline:		
Calculate baseline	Peak Window Accept negative peaks	
Baseline Settings	Reject Peaks Pea <u>k</u> skim 10.00 ra	tio
	Colu <u>m</u> n height (bed height): 0.00 cm Column <u>V</u> t: 0.00	
@	Save and Edit Peak Table DK Cancel	

3) 点击积分窗口中的 OK, 查看积分结果



积分结果如下图显示,Peak data 中默认显示保留时间、面积和高度



4) 如果需要查看更多的数据,可以在 Peak data 区域点击右键,选择 Customize

Pe	eak data	Integration sur	nmary				
N	٩o	Peak name	Retention (min)	Area (min*mAU)	Height (mAU)		Save Column Performance Statistics
Þ	1		1.000	28.5752	93.691		
	2		1.932	67.5762	133.905		Copy to Clipboard
						. /*	Edit Peak Table
						1	Customize

在 Select peak table columns 对话框中找到需要显示的数据,如 percent of total peak area、plate height(HETP)、Asymmetry等, 如需对峰用阴影填充,选择 Fill peaks 复选框,并选择填充阴影类型 和颜色(Color),点击 OK 执行



Peak data 区域中将会显示增选的分析结果

1	Peak	data 📗	Integration sur	nmary						
	No		Peak name Retentio		Area (min*mAU)	% of total peak area	Height (mAU)	Plate height (cm)	Plates per meter	Asymmetry
	۲.	1		1.002	31.9413	29.68	97.344	0.04544	2200.5	2.09
		2		1.932	75.6955	70.32	141.203	0.03637	2749.7	1.77

6.4 生成层析报告

 如果想将层析结果以报告的形式打印或输出出来,请在工具栏中点击 报告按钮

<mark>∧</mark> ∎ E	👍 Evaluation - [Manual Run 005]										
١	File	Edit	View	Integrate	Operations	Procedures	Tools	Help			
	7 🏝	1 💾	🗖		6	3	J.		Λ	XX	

2) 选择合适的报告模板,单击 Preview

Generate Report	×
<u>F</u> ormat	
(Global) Chromatogram (Global) Chromatogram including logs	<u>N</u> ew
(Global) Chromatogram including hogs (Global) Chromatogram including peak table (Global) Chromatogram including pH curve	Delete
(Global) Full chromatography report (Global) Full chromatography report including pH cui	<u>E</u> dit
	E <u>x</u> port
	Import
Pre <u>v</u> iew	Close

也可以点击 Edit,在弹出的自定义窗口中通过工具栏中的功能按钮, 编辑文字、图片、图谱以及层析数据

Customize Report: Chromatogram including peak table	- 🖻 🔀
File Edit Wew Insert Layout Help	
Preview Prev Page Next Page One Page 51% 👱 Add Page Delete Page Exit	
Page 1-2 of 2 Edk Mode	

3) 将显示的报告打印出来即可,点击右边的 Exit 退出报告模式

	Customize	Report:	Chrome	atogram i	includ	ing pea	k table			
	File Edit	View	Insert	Layout	Help					
1	Preview	Prev Pag	je Next F	^D age One	Page	51%	•	Add Page	Delete I	Page Exit
	🖻 📂			6	Ê		T	1		

- 6.5 色谱曲线叠加比对
 - 1) 首先打开一个层析结果,通过编辑使其只显示待比较的曲线

Ag Eve	Iluation - [Exar	nple Result001*]	
	lle Edit Yew	Integrate Operations Brocedures Tools Help _ @	×
1	ħa 💾 🖥] 🖨 [🗞 🗅 🛍 [🤊] 🖂 🔛 📈 💷 📴	
	1		
Result Newlyston	RALU 480 440 440 442 400 300 300 300 300 300 200 100 140 140 140 140 140 140 140 140 1		
	A: U/1_215nm@0	TPEANT	
14	Dash data in a		

2) 需要与其他色谱图曲线进行叠加对比时,在 File 下拉菜单中选中 Open to Compare,点击 Curves

^ <u>∎</u> 8	valu	ation - [Manual Run O	06]		
۵	File	Edit View Integrate	Oper	rations Procedures Tool	s I
*	5	New	•	h 🛍 🍋 🗔	
	1	Open	•		
	1	Open to Compare	•	Chromatograms	
Resu	<u>11</u>	Multi Result Peak Compare.	Curves		
JE Na		Close			nual I Run
aviga		New Folder		Train 200 On On Think and	a rear
ē,		Save Ctrl	+5		
		Save All			
		Save As			
		Sign Result			
	≓	Print Ctrl	+P		
	As.	Report Ctrl	+R		
		Export	•		
		Import	•		
	-	Log Off 'Default'			
		Exit UNICORN			

3) 在弹出的对话框中点击 Browse 寻找需要比对的结果,选择曲线显示 方式,点击 OK 完成色谱图曲线比对

Open Curves to (Compare				×						
Curve selection			_								
Folder:	/DefaultHome/Dem	~	Browse								
Result	Example Result002		~	Browse	All						
Chromatogram:	1		~	Browse	All						
Curve:	UV1_215nm		~	Browse	All						
Found curves Search Clear Select All	Found curves Search Dear Select All										
Curve options Ouve options O Qverlay Stack Mirror											
()		919 9		ОК	Cancel						

结果如图



7. 退出程序并关机

- 1) 在任务栏中点击 Administration 窗口,主菜单 File 下拉框中选择 Exit UNICORN
- 2) 关闭计算机,并关闭 ÄKTApure 主机电源

III. Demo 实验:使用脱盐柱进行蛋白缓冲液的置换

1. 实验目的

- 使用脱盐柱对细胞破碎后的表达上清液进行缓冲液置换,使样品能更有 效地结合在离子交换柱上
- 学习使用程序编程进行实验

2. 实验材料

- 层析柱:5 ml Hitrap desalting column 2 根
- 缓冲液: 20 mM Tris-HCl, pH 8.0
- 其余溶液:去离子水;20%乙醇水溶液(v:v)
- 实验耗材:5 ml 注射器,2 ml 样品环,15 ml 离心管
- 待分离蛋白样品:大肠杆菌表达的带 6 X His 标签的重组绿色荧光蛋白
 GFP (His-GFP),经 0.22 µm 针头滤器过滤

3. 实验步骤

3.1 开机

1) 先打开 ÄKTApure 电源,控制面板上的 power 灯稳定不再闪烁时,打 开电脑,双击打开 UNICORN 6 软件

📲 Log On - UNICORN 🛛 🛛 🔀										
Use <u>W</u> indows Authentication										
<u>U</u> ser Name:	Default 💌									
<u>D</u> omain:										
Access Group:	Administrators									
0	<u>OK</u> <u>C</u> ancel Options >>									

2) 点击 OK 进入软件,打开系统控制 (System Control) 窗口

R 9	System	n Contro	ol												
Ð	e <u>E</u> d	it ⊻iew	v <u>M</u> anu	al ⊇ystem <u>T</u> o	ools <u>H</u> elp										
Ľ						💷 🍢 -									
2	0	akta pur	re												-
Met	RL	un Data													• ×
NDON	Syste	m state		Acc. volume	Sy	stemflow	Conc B	PreC pressu	re DeltaC pres	ure UV1_2	80 (Cond	pH		
Church	Ready			0 ml	0.0	100 ml/min	0.0 %	0.00 MPa	0.00 MPa	0.000 m	AU [1	10.23 mS/cm	Off		
stor															
	0	hromato	gram												• ×
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	-40	°													unin
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	Pn	ocess Pict	ture												• ×
		0	0.000	ml/min 0.0	%в				PreC 0.00 MPa	0.000 mAU	10.23 mS/	/cm pH Off			
								C	DeltaC 0.00 MPa						
		Buffer i	inlets	Pumps			Manual load @	_							
		1			,	Mixer valve	manual road	02							
	-	= A1	L	A			E	W1	Column			pH valve	Outle	t Frac	
					-			Col		107	Grad	- Burning		A ATT	
		100			-	IVIIXEF	IVI Syp		By-pass	= 00 =	Cond	Restricto	- w	- Heres	9
	_	B1		в		_							- -	0	
								-1							
	- 0								- Constitution						
		Ready	6	lock: Nowa	kch ∣0	Connection = Conne	cted in control Contr	olled by Default(9HCA	-SYYSDV1						

- 3.2 泵冲洗
 - 1) 将缓冲液管 A1 插入到 20 mM Tris-HCl, pH 8.0 缓冲液中,在 System Control 窗口选择 Manual > Execute Manual Instructions

a s	System (Control						
Fil	e Edit	View	Мал	iual	System	Tools	Help	
📥 🕟 🖱				Exe	cute Manua	al Instruct	tions	Ctrl+M
			\odot	Hold	1		Ctrl+	-Shift+H
2	🗌 🔿 ak	ta pure		Pau	se		Ctrl-	+Shift+P
Meth	Runi	Data		Сог	itinue		Ctrl+	-Shift+C
od Na	System	state		End			Ctrl-	+Shift+E

2) 在跳出的在 Manual instruction 窗口中选择 Pumps >Pump A wash,选择 Inlet 为 A1,点击 Execute,进行泵冲洗

Manual instructions - akta pure		
Instructions: Pumps System flow Gradient Pump B wash Mixer by-pass wash System wash Flow path Monitors Fraction collection Alarms Wash settings	Selected column type: Select	Instruction execution list. Insert Delete
Save result as:	<u>B</u> rowse	Execute Close

3.3 安装柱子

在 Manual instruction 窗口中,选择 Flow path > Column position,按
 连接需要选择柱位,例如选择 Position 2,选择溶液流向为 Down flow,

点击 Inse	ert		
Manual instructions - akta pure			
Instructions: Pumps Flow path Inlet A Inlet B Injection valve Column positon pH valve Outlet valve Mixer valve Injection mark Monitors Fraction collection	Selected column type: Parameters for Column position 2 Flow Direction ③ Down flow	Select Instruction exect Instruction exect Insert Column position w	ution list: Delete 2. Down flow
Save result as:	un	Browse	Execute Close

2) 在 Manual instruction 窗口中,选择 Pumps > System flow,设置 Flow rate为 0.5-1 ml/min,点击 Insert

Manual instructions - akta pure						
Instructions: System flow Gradient Pump A wash Mixer by-pass wash System wash System wash Flow path Monitors Fraction collection Alarms Wash settings	Se P	elected column type:	[0.000 - 2 1] 🛢 ml/min	5.000) Linear Flow	Instruction execution list: Insert Delete Column position 2, Down flow System flow 1.000, Off)
Save result as:	ing run			Browse		Execute Close

3) 在 Manual instruction 窗口中,选择 Alarms > Alarm pre column pressure,设置 High alarm 为 0.3 MPa,点击 Insert,然后点击 Execute 执行

Manual instructions - akta pure				X
Instructions: Flow path Monitors Fraction collection Alarm system pressure Alarm delta column pressure Alarm UV1 Alarm pre column pressure Alarm pre column pressure Alarm pre column pressure Alarm pre column statistication of the s		Selected column type: Parameters for Alarm pre column pressure Mode Disabled High alarm 0.0 2 - 20.00] 0.00 # MPa Low alarm 0.00 # MPa	Select	Instruction execution list Insert Delete Column position 2, Down flow System flow 1000, Off Alarm pre-column pressure Enabled, 0.50, 0.00
Save result as:	ring ru	ın	Browse	Execute Close

- 4) 在柱位阀 2A 口连接上一根 PEEK 连接管
- 5) 待连接管的出口有持续的液体流出且无气泡的情况下,除去层析柱的 上堵头,将层析柱柱头与连接管出口相连,但不要拧紧
- 6)除去层析柱下堵头,待层析柱出口有持续的液体流出,且无气泡的情况下,将层析柱出口连接到柱位阀的2B口上
- 7) 拧紧上接头,将流速调整为 5 ml/min,对层析柱进行平衡至少 5 个柱 体积
- 8) 平衡完毕后,单击 Pause 暂停

🖶 Sys	🝓 System Control								
<u>F</u> ile	<u>E</u> dit	⊻iew	<u>M</u> anual	<u>S</u> ystem	<u>T</u> ools	<u>H</u> elp			
			II D			M			

3.4 样品环的安装与清洗

在上样阀的 LoopF 与 LoopE 口连接上 2 ml 的样品环,用注射器抽取缓冲 液,连接到上样阀的 Syr 口上,推入缓冲液以清洗样品环,可重复几次

3.5 上样

用注射器吸取约 2.5 ml 的样品, 排除气泡后,连接到上样阀的 Syr 口上, 将注射器内的样品推入,并将注射器保留在上样阀上

3.6 准备收集器

在收集器内按顺序放入一定数量的 15 ml 收集管,调整悬臂到 1 号位

- 3.7 运行实验-手动命令
 - 在 Manual instruction 窗口中,选择 Flow path > Injection valve,上样 阀选择为 Inject 状态,点击 Insert

Manual instructions - akta pure		
Instructions: Pumps Flow path Inlet A Inlet B Injection valve Column position pH valve Outlet valve Mixer valve Injection mark Monitors Fraction collection	Selected column type: Selected column type: Selected column type: Selected column valve Position Inject	Instruction execution list:
Save result as:	lg run	wse

2) 在 Manual instruction 窗口中,选择 Flow path > Outlet valve,选择出 口阀位为 Frac

Manual instructions - akta pure	
Instructions: Pumps Flow path Inlet A Inlet B Injection valve Column position pH valve Outlet valve Mixer valve	Selected column type:
Injection mark Monitors Fraction collection Save result as: Autourpdate of parameters duri	n Execute Close

3) 在 Manual instruction 窗口中,选择 Fraction collection > Fractionation, 设置 Fraction size 为 2 ml,点击 Insert

Manual instructions - akta pure		
Instructions: Fraction collection Fractionation Stop fractionation Peak fractionation Stop peak fractionation Reset frac number Fractionation in outlet valve Stop frac in outlet valve Peak fractionation parameters Peak fractionation parameters	Selected column type: Select.	Instruction execution list: Insert Delete Injection valve Irject Dullet valve Frac Fractionation Volume, 2.00
Save result as:	growse.	Execute Close

4) 在 Manual instruction 窗口中,选择 Pumps > System flow,设置 Flow rate 为 5 ml/min,点击 Insert

Manual instructions - akta pure		
Instructions: Pumps System flow Gradient Pump A wash Pump B wash Mixer by-pass wash System wash Flow path Monitors Fraction collection Alarms Wosh settings	Selected column type: Select Parameters for System flow Flow rate 0.000 - 25.000 Flow rate 5 ml/min Linear Flow Pressure control Off System flow 5.000, Dfr System flow 5.000, Dfr)
Save result as:	Browse	Execute Close

5) 在 Manual instruction 窗口中,点击 Save result as 后面的 Browse

Manual instructions - akta pure	
Manual instructions - akta pure Instructions: Pumps System flow Gradient Pump A weah Pump A weah Pressure control Dff Flow path Monitors Flow path Adarms	
Save result as: Browse Image: Constraint of parameters during run Execute	Close

6) 在弹出的对话框中选择结果存储位置,输入文件名,点击 OK

Select Resu	lt Name & Locatio	n		
🝃 📹	Results, Folders	• •		
Folder name		System	Last modified	Created by
😑 📑 HCA-5	5YYSDV1			
🗉 📄 De	efaultHome		2/4/2013 9:56:48	System
H 🗧	akta pure (Manual)		2/4/2013 11:14:5	Default
8 🧧	Demo		2/21/2013 2:54:1	Default
	🔊 Demo 001	akta pure	2/21/2013 3:02:4	Default
<				
Name: De	mo Run			
۲			ОК	Cancel

7) 点击 Execute 执行上述命令

Manual instructions - akta pure		×
Instructions: System flow Gradient Pump A wash Pump B wash Mixer by-pass wash System wash Elow path Monitors Fraction collection Alarms Wash settings	Selected column type: Parameters for System flow Instruction execution list Flow rate [0.000 - 25.000] 0.000 = ml/min Linear Flow Pressure control Injection valve Inject Off System flow 5.000.00f	
Save result as: /DefaultHome/Demo/Demo	Run Browse un Execute Close	

8) 约 3 min 后,待紫外与电导峰均出现后,点击工具栏中的 End,停止 实验

🖶 Sys	stem C	ontrol					
Eile	<u>E</u> dit	⊻iew	<u>M</u> anual	<u>S</u> ystem	<u>T</u> ools	Help	
			II			M	

3.8 运行实验-Method Wizard 编程

1) 在任务栏中点击进入 Method Editor 窗口



2) 在工具栏中点击 Create a new method, 启动方法编程

💕 Me	thod	Editor								
File	Edit	View	Phases	Tools	Help					
1		þ, I		So		n 9	6		Ê K.	System:

3) 在 Predefined Method 下拉框中选择层析实验的类型 Desalting (DS)

New Method
System:
AKTA pure 🔹
Create a new method by using the:
Predefined Method:
Desalting (DS)
◎ Empty Method:
Method Description
After equilibration and sample application the proteins are eluted isocratically. This technique is commonly used for buffer exchange.
OK Cancel

4) 点击 OK, 方法编辑界面中出现预设的 DS 层析实验各个阶段及参数

	Phase Properties	Text Instructions			
Method Settings	Method Settings				
Equilibration Sample Application	Column selection Show by technique Desalting Column type Any Show only suggested columns Column volume 0.100 ml Pressure limit pre-column 2.00 MPa [0.02 - 20.00] V Use flow restrictor				
Elution	Column position By	-pass 🔹			
	Flow rate	1.000 ml/min [0.000 - 25.000] Control the flow to avoid overpressure			
	Inlet A A1 Inlet B B1	• •			

5) 在 Method Settings 编辑界面中,选择实验所用的层析柱 Hitrap desalting column

Column selection	
Show by technique	Desalting -
Column type	HiTrap Desalting

6) 选择柱子连接到柱位阀上的位置以及缓冲液入口

Method Settings				
Column selection				
Show by technique	Desalting]	•	
Column type	HiTrap D)esalting	•	
V Show only sugge	Show only suggested columns			
Column volume		5.027	ml	
Pressure limit pre-c	olumn	0.50	MPa [0.02 - 20.00]	
Use flow restricto	r			
Column position 2			•	

对于选择的预装柱,系统会预设好默认流速,也可以在一定范围内进 行修改

7) 选择检测的 UV 波长为 280 nm

Monitor settings						
UV variable wavelengths						
UV 1	280	[190 - 700]	nm			
🔲 UV 2	254	[190 - 700]	nm			
🔲 UV 3	214	[190 - 700]	nm			

8) 点击 Equilibration 进入平衡阶段设置,输入平衡体积 2 CV

Equilibration				
$\ensuremath{\overline{\mathbb{V}}}$ Reset UV monitor (recommended if the equilibration of	occurs befo	re the purification).		
☑ Use the same flow rate as in Method Settings	🔽 Use th	ie same inlets as ir	Method	d Settings
Flow rate 5.000 ml/min [0.000 - 25.000]	Inlet A	A1	•	
	Inlet B	B1	•	0.0 % B [0.0 - 100.0]
	🔽 Fill the	e system with the s	elected	buffer
Equilibrate until				
the total volume is 2.00 CV				

9) 点击 Sample Application 进入上样阶段设置,选择上样的方式为 Manual Load,输入清空样品环的体积为4 ml

Sample Application							
Use the same flow rate as in Method Settings Flow rate 2 ml/min [0.000 - 25.000]							
Inject sample from loop	Fill the loop using	Manual load 🔹					
Ising a start of the start o	Loop type	Capillary loop 👻					
Inject sample directly onto column	Loop position	1					
	Sample inlet	A2 -					
	Fill loop with	0.60 ml					
	Empty loop with	4.00 ml					

10) 点击 Elution 进入洗脱设置,首先选择洗脱的流速、缓冲液入口以及 缓冲液流向

Sample Application							
Use the same flow rate as in Method Settings Flow rate 2 ml/min [0.000 - 25.000]							
Inject sample from loop	Fill the loop using	Manual load 🔹					
	Loop type	Capillary loop 👻					
Inject sample directly onto column	Loop position	1					
	Sample inlet	A2 -					
	Fill loop with	0.60 ml					
	Empty loop with	4.00 ml					

选择 Isocratic elution 方式,设置收集体积为 2.5 CV,使用 Fraction collector 进行固定体积收集

Isocratic elution								
Volume 2.50	CV 0.0 % B [0.0 - 1	00.0] Fill the system with the selected buffe	er -					
Gradient elution								
Start at 0.0 %	6 B [0.0 - 100.0]	Fill the system with the selected buffer						
Target %B Length (0-100) Linear 100.0 20.00 Add Segment Delete Segment Delete Segment								
Fractionate	Fractionation settings							
in waste (do not collect)	Fractionation type	Fixed volume fractionation	ed :					
outlet valve	Fractionation destination	Setting:	s					
 using fraction collector 	Peak fractionation destination	Peak Fr	rac s					
Fraction collector	Fixed fractionation volume	2.00 ml [0.00 - 50.00]						
	Peak fractionation volume	2.00 ml [0.00 - 50.00]						

11) 完成所有设置后点击工具栏中的保存按钮

🖻 M	ethod	Editor -	UNTITLE	D*							
Eile	<u>E</u> dit	⊻iew	Phases	<u>T</u> ools	Help						
1	1	a .		to		ĥ	7	6	L . 🖻	Ê	1

选择保存的路径,输入方法名称后 Save

Save As				×
اگ 📹	Methods, Folders	• .		
Folder nam	ne	System	Last modified	Created by
😑 📑 НС	A-5YYSDV1			
🛛 🖻 📄	DefaultHome		2/4/2013 9:56:48	System
	🚞 akta pure (Manual)		2/4/2013 11:14:5	Default
<				>
Name: [Demo			
System:	akta pure			*
0			Save	Cancel

12) **如要运行已创建的方法**,在 System Control 窗口 Method navigator 中



选择方法,右键点击 Run

在跳出的 Start Protocol 中确认设置的项目,点击 Start 开始运行

esult Name and Location >>	
	Date: 2/19/2013 5:32:17 PM +08:00
	User: Default
	Method: Demo
	Add unique identifier to result name
	Directory:
	/DefaultHome Browse
	Scouting subdirectory:
	Demo
	Name:
	Demo 001
	<back next=""> Start Cancel</back>

- 4. 结果分析与讨论
 - 1) 切换到 Evaluation 窗口中,选择保存的结果并打开

_								
∧ ∎	Evaluatio	on						
E	ile <u>E</u> dit	⊻iew	Integrate	Operations	Procedures	<u>T</u> ools	Help	
2	5 📶	FF I	8	6		7		📈 🛋 📑
	Result Na	vigator					+= ×	
л	Besults	Recent	Runs Fin	d Besults				
esi	Troodito	s		arroodito				
JEP	Ref	resh	Preferen	ices				
VaV			\sum					
igal	Rem	iove	Remov	e All				
Q,								
	₽ . <mark>₩</mark>	Manual I	lun 003 (/DefaultHom	ie/akta pure	(Manual	ມ	
		Demo O(1 (/Defau	lltHome/Dem	o)			
		Manuall	?un 002 (/DefaultHom	ie/akta pure	(Manual)) 🛛	
	i ∎	Manual I	Run 001 (/DefaultHom	e/akta pure	(Manual	ນ 🗌	
	- <u>- </u>	Manual I	Run 010 (/DefaultHom	e/akta pure	(Manual	n	
	1 - 5	Manual I	Run 009 (/DefaultHor	e/akta pure	íManual	n	
	128	Manual	3un 008 (DefaultHor	e/akta pure	(Manual	n l	
		Manuali	1un 000 (DefaultUer	e /akta pure	(Manual	,, ,,	
	P 44	manuali	140 007 U		ezakta pure	(manual	11	
	<u>ا</u>	Manual I	łun 006 (/DefaultHom	e/akta pure	(Manual	11	

- 2) 现场讨论:
- 紫外与电导峰分别代表什么?
- 哪些收集管中包含样品?
- 5. 清洗与储存

程序运行结束后需要手动清洗系统和层析柱并保存将二者保存在 20%乙 醇中。

 1) 将缓冲液 A1 的入口放入经脱气的去离子水中,进行泵清洗(见 2.2.3),待泵清洗结束后,用去离子水清洗柱子:在 Manual instruction 窗口中,选择 Pump > System flow,设置 Flow rate 为 5 ml/min,点击 Insert

Manual instructions - akta pure	
Instructions: System flow Gradient Pump A wash Pump B wash Mixer by-pass wash System wash Flow path Monitors Fraction collection Alarms Wash settings	Selected column type:
Save result as: //DefaultHome/Demo/Demo R	n Browse

2) 在 Manual instruction 窗口中,选择 Alarms > Alarm pre column pressure,设置 High alarm 为 0.3 MPa,点击 Insert,然后点 Execute

Manual instructions - akta pure	
Instructions: Flow path Monitors Fraction collection Alarm system pressure Alarm delta column pressure Alarm per column pressure Alarm UV1 Alarm conductivity Alarm pH Alarm intel & ai sensor Alarm intel & ai sensor	Active column type: Any <u>Select</u> Parameters for Alam delta column pressure Mode Disabled © Enabled High alarm [0.02 - 20.00] 20.00 MPa Low alarm [0.00 - 20.00] MPa
Save result as: Image: Save result as: Image: Save result as: Image: Save result as: Image: Save result as:	Browse n Execute Close

3) 在 Manual instruction 窗口中,选择 Flow path > Column position,选择 Position 2,选择溶液流向为 Down flow,点击 Execute

Manual instructions - akta pure		
Instructions: Pumps Flow path Inlet Å Inlet B Injection valve Column position pH valve Outlet valve Mixer valve Injection mark Monitors Fraction collection	Selected column type: <u>Select</u> Parameters for Column position Position 2 Flow Direction Down flow Up flow	
Save result as:	Browse	Close

- 4) 运行5倍柱体积后,暂停
- 5) 将缓冲液 A1 的入口放入经脱气的 20% 乙醇水溶液中,进行泵清洗
- 6) 待泵冲洗结束后,机器会自动继续之前 5 ml/min 的流速,用 20% 乙 醇水溶液清洗柱子

- 7) 待运行 5 倍柱体积后,先拧松柱子上接头,将下接头从柱位阀上拧下, 并拧上柱子的下堵头
- 8) 拧下柱子上面的接头并拧上柱子的上堵头
- 9) 在 System Control 界面命令栏中点击 End, 结束清洗

🖣 Sy	stem C	ontrol					
Eile	<u>E</u> dit	⊻iew	<u>M</u> anual	<u>S</u> ystem	<u>T</u> ools	<u>H</u> elp	
						M	-

10) 在任一 UNICORN 6 软件窗口中点击 File > Exit UNICORN 退出软件

E	🝓 System Control									
	File	Edit	View	Manual						
ſ	►	Run Ctrl+R								
9	1	Open Ctrl+O								
k	6									
	Recent Method Runs									
	🐔 Log off 'Default'									
1		Exit UNICORN								

11) 关闭 ÄKTApure 电源

Ⅳ. 日常维护

1. 每日维护

- 1)系统(System)
- •清洁擦拭外表,防止试剂或结晶的盐腐蚀设备
- •使用完毕,须用水将系统冲洗干净,之后再用20%乙醇清洗系并保存所有的流路

2) pH计(pH electrode)

- •使用前校正pH计(一定要先校正pH7)
- •用后将pH计拆下放入保护液(1:1 pH 4 buffer和 1 M KNO3,或pH4.0的饱和KCl 溶液)。

3)系统泵(Pump)

• 检查泵头周围是否渗漏,如果泵头有渗漏或是流量不准确,采取相关措施解决。

•更换缓冲液时,需排尽泵头里的残存气泡,否则会影响流速的准确性。

2. 每周维护

1) 在线滤器(On-line filter)

•清洗过滤片,如有必要须更换过滤片,否则会形成很高的在线压力,流速降低。

2) 缓冲液筛网(Inlet filters)

- •检查溶液入口的筛网是否很脏,如有必要须更换。
- 3) 泵后腔冲洗系统
- •更换泵后冲洗液(20%乙醇)。
- •如果冲洗液瓶中液体量增加,说明泵头密封圈渗漏,须更换。
- •如果冲洗液不循环,说明单向阀堵塞或损坏,需要清洗或更换。

3. 每月维护

1) 压力检测器 (Pressure)

•零点校正 (pressure offset)。

2) 紫外监测器(UV Monitor)

• 用注射器推10%的表面活性剂(如 Decon90,Deconex11,DBS25, SDS等)注入 紫外流动池,停留20分钟,用水冲洗。

•用注射器推甲醇或1M NoOH注入紫外流动池,停留20分钟,用水冲洗。

3) pH/电导检测器(pH/C Monitor)

• 清洗流动池: 拆下pH电极,用1M NaOH清洗pH和电导流动池 30分钟,用 水 冲洗。

4) 系统清洗 (System cleaning)

• 按时清洗系统或在遇到问题时清洗系统。用1M NaOH 或甲醇执行SystemWash 指令,之后立即用水将NaOH或甲醇冲洗干净。