

## Malvernpanalytical Microcal PEAQ-ITC 中文簡易操作流程

Made by Roy of DKSH Taiwan Ltd.

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Market State A State

頁1/10



1. 開啟電腦

2. 開啟位於儀器右後方的開闢



3. 點選桌面上的 開啟實驗操作軟體,下方視窗將會彈出,並確認位於儀

器前方的燈示為綠色確保軟體和儀器已相連



 確保自動清洗區域放置的血清瓶已填滿約一半以上的適當溶劑(蒸餾水、甲醇 及清潔劑 ex: 20% Contrad 70 或 14% Decon 90),此外亦須檢查廢液瓶及溢流 瓶是空的,可參考下方圖表



Part	Description				
1	Cell Cleaning Tool				
2	Detergent bottle				
3	Methanol bottle				
4	Water bottle				
5	Waste bottle				
6	Overflow bottle				

- 5. 確認 reference cell 已充填蒸餾水,並留意每周更換一次(確保避免細菌滋生)
- 6. 在下方視窗左側進行包含樣品濃度在內的參數設定



Malvern MicroCal	PEAQ ITC CO	ontrol Software					01			
Ran Devertment M	laintonance De	sign Experiment	A 1.4							
Run			And Run	Deat 3						
Experiment Information	on	¢						-0	0-0	2
Part Ma parts Ma Lowneet	8	於該位置	進行樣。	品濃度設定		-	Setting feetperature	Caulibiding the	ang anti ka	
Instrument Settings		1	0.02-							
hempes asses (*C) this separativestal anothest injection, followed by LR 2	25.0 with make a single th 7 pi injections.	ーー 能 諸	選後可 、轉速 定回穩	設定溫度、refe 、起始延遲時間 時間、滴定次數	rence power 1、 滴定間隔 、 滴定 體積	・回顧 寺間、 奪參數				
The following settings w	the used		0.005-							
Reference Power Galifia	10.0	(5/)	-	建議設定:						
antipad	High	Edd	0-	Reference po	wer: 5 µcals/s					
the Street (spec)	750	) de		Feedback: hig	th					
tertial Certaiy 53	60	-	-0.005-	Stir speed: 75	0 rpm					
III (minut room of	150		-001-	Initial delay:	50 s					
Apartices Duration (st	4		-	Spacing time:	150 s					
			-0.015	Duration time	.45					
				Number of in	iections: 19					
			-0.02-	Injection volu	me: 2 ul					
			-0.025-	injection void	ine. z pr					
			1	. <u>1</u> .0.4	-63	-0.2	-0.1	Time (min)	0.1	to

 通常來說此時系統是乾淨的,但若擔心可以在此時點選下方示意圖的 Clean 按鍵並依序點選 next 進行清潔

Malvern MicroCal PEAQ-I	IC Control Software		01
Run Experiment Maintenance	Design Experiment		
Clean	Load	Run Dean 🔕	
		0	0-0-0-0-0
		() Introduction	
			To view a video depicting all the clearung steps club the stay button.
			To enter the Clima Instrument woldbare, dios sent
		Choose Cleaning Method(s)	
		Insert Cell Cleaning Tool	
		M Attach Fill Port Adapter	
		Move Pipette to Clean Location	
		Detach Fill Port Adapter	
		Remove Cell Cleaning Tool	



8. 按完 next 鍵後,下個步驟是選擇清洗的模式,通常使用預設值即可(參見下圖),接著依序按下每個步驟右下角的 next 按鍵,完成總計6步驟的清潔程序

Choose Cleaning Method(s)	
Cell Cleaning Method	Syringe Cleaning Method
Rinse Rinse with water.	Rinse     Rinse with water, then dry using methanol.
Wash Wash with detergent, then rinse with wash	Wash Wash with detergent, rinse with water, then dry using methanol.
Soak Soak in detergent for 30 minutes at 60 ° then rinse with water.	C None
None	
Back	Next

- 清洗的最後一個步驟將提示您移除清洗用具,按下右下角的 Done 完成清洗, 並按下 Run 按鍵回到實驗執行頁面準備開始設定各個實驗參數
- 10. 在實驗設計處(Run 頁面的左側,參見下圖)進行參數設定,預設的方法為 CaCl2/EDTA 滴定實驗,使用者請先將下圖紅框處的濃度進行修改

Run Experiment Information [Syr] (M) [Cell] (M) IO0e-3 IO0e-6 Comment Comment I I Instrument Settings Temperature (°C) Z5.0 This experimental method will make a single o injection, followed by 18, 2 µL injections. The following settings will be used: Reference Power (µcal/s) S.00	ан а 14 µL	<	Load 0.0 0.0	Run 125 - - - - 	Clean	0
Experiment Information	А 14 µL	<	0.0 0	125 - - - - - - - - - - 		
[Syr1 (M)     1.00e-3       [Cell] (M)     100e-6       Comment     1       Instrument Settings     25.0       This experimental method will make a single 0 injection, followed by 18, 2 µL injections.     Store 100 minutes 100 m	).4 µL		0.0 0.0	02-		
[Cell] (M)     100e-6       Comment     I       Instrument Settings     I       Instrument Settings     25.0       This experimental method will make a single of injection, followed by 18, 2 µL injections.     I       The following settings will be used:     Reference Power (µcal/s)     5.00	).4 µL	•	0.0 0. 0.0	02-		
Comment	).4 µL		0.0 0.0 0.0	025- - - - - - - - - - - - 		
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Instrument Settings           Temperature (°C)         25.0           This experimental method will make a single ( injection, followed by 18, 2 µL injections.           The following settings will be used:           Reference Power (µcal/s)         5.00	).4 µL	¢.	0.0	15-		
Temperature (°C)     25.0       This experimental method will make a single ( injection, followed by 18, 2 µL injections.       The following settings will be used:       Reference Power (µcal/s)     5.00	).4 µL		0.0	15-		
This experimental method will make a single ( injection, followed by 18, 2 µL injections. The following settings will be used: Reference Power (µcal/s) 5.00	).4 µi.					
The following settings will be used: Reference Power (µcal/s) 5.00			0	.01-		
Reference Power (µcal/s) 5.00				-		
			(s 0.0	05-		
Feedback High			cal/	1		
Stir Speed (rpm) 750			Ľ,	0-		
Initial Delay (s) 60			DP 00	05		
Injection Spacing (s) 150			-0.0	]		
Injection Duration (s) 4.0			-0	01-		



11. 可以在 comment 欄位中填入跟樣品有關的描述, ex: cell: EDTA, syringe: CaCl2

Experiment Info	ormation	<
[Syr] (M)	1.00e-3	
[Celi] (M)	100e-6	

- 12. 點選下圖紅框處的鉛筆圖示,針對滴定條件進行設定,建議設定可參照第六
   步驟示意圖,注意:第一針滴定為平衡用,體積請改設為 0.4μL
- 13. 點選另存為方法將目前設置的方法保存以供日後使用, ex: EDTA method.itcm, 然後點選保存, 便會將該方法保存在預定義的方法文件夾中
- 14. 在開始實驗前,我們必須先將 sample cell 和 syringe 中的樣品填充完畢,點選 下示意圖的 load 按鍵並依照指示影片一步步完成樣品填充





15. 在填樣開始前請使用 buffer 清洗 sample cell,再放入樣品,這可以使實驗後的接合位數(stoichiometry)較接近真實狀況,下面我們以 EDTA 溶液舉例說明



- a. 將 loading syringe 輕輕地插入 sample cell 觸及底部,並將殘餘溶液移除
- b. 準備 EDTA 溶液於 loading syringe 中約 300μL,注意過程中盡量不要產生 氣泡
- c. 將 loading syringe 輕輕地插入 sample cell 觸及底部,然後將其抬起 1-2mm 然後緩緩地將 EDTA 溶液注入並抽吐清洗約兩次
- d. 盡可能地去除 EDT 溶液
- 16. 將樣品注入 sample cell,此步驟中可以準備約 350μL,在注入樣品後觀察 loading syringe 刻度以確保 sample cell 填充確實而無產生氣泡在其中
- 17. 確認參考樣品槽填充乾淨的水,如若需要更換則參考上述填樣流程回填乾淨 的水於參考樣品槽
- 18. 將端口適配器(port adaptor)插入 Syringe 的孔洞並點選視窗右下角的 next,這 會促使柱塞向下移動準備抽樣





19. 首先放置一已裝載 60µL 樣品的微量離心管(PCR tube)於下圖紅框處,接下來 將 Syringe pipette 移至填樣位置,點選 next 開始抽樣



- 20. 待樣品填入 Syringe 完成後將其放在 storage 位置,移除端口適配器,點選 next
- 21. 將 Syringe 放入樣品槽中,點選視窗右下角的 done 並回到 run 頁面準備進行 實驗



22. 點選下圖的 start 按鍵開始實驗,實驗時間取決於滴定數及間隔時間設定







23. 實驗結束後數據將會自動存取,如若不繼續進行實驗,則如同步驟7一樣點 選上方 Clean 鍵開始逐步清洗流程,之後我們可以關閉軟體前往分析軟體進 行數據處理



- 24. 分析數據,首先打開位於桌面的分析軟體快捷鍵
- 25. 在初始畫面的 Start Analysis 作業區,從 Experiment 分頁中選擇欲分析的數據 並開啟,可參見下圖

nałyze Experiment(s) De	sign Experiment	
art Analysis	Start Analysis Ove	New Admentantes Amon Controls   Admental Preventation   1 Building
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Browse <u>C1</u> > <u>Distribu</u>	tion > PEAQDEAP-Documents > E	Experiments > Getting Started 😁
Browse CA > Distribut	tion > PEAQDEAP-Documents > E	Preview - CaEDTAGetStart.itc
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26. 在點選完 open 後軟體將完成對數據的分析,並開啟下圖的 Overview 分頁, 於此您可以由左方點選複數筆數據進行數據比較,舉例如下方第二張圖片, 注意只有在分類上被歸類為有反應的"binding"才會進行數據擬合,歸類 為"Control"則不會







27. 可在 Overview 視窗中的上方下拉選單選擇樣品間的結合位數,重新擬合

	lysis	Overview	Adjust B	aseline	Assign
Fitting M	1odel	One Set of Site	s		~
		One Set of Site	28		
		Two Sets of Si	tes		
	10 -	Dissociation			-
	9.5	Sequential Bin	ding Sites		
	9	One Set of Site	es - SIM		
-	8.5	Enzyme Kineti	cs - Multipl	e Injectio	ins
I/s	8	Enzyme Kineti	cs - Single	Injection	
0		1	1	H	I

28. 點選上方視窗的 presentaiton,會開啟新的分頁選項,其中包含有分析完的數 據表格及用以呈現最終結果的 Final figure

Presentatio	n		Deriver i	Nerview Ada	int Easeline	Ausgr	Controli	Adjust 7	Free	ntetor		1140											
Terror Table Find Figu	n 50	attie Plot	injection Table Sta	haboi Piliti S	grature Pic	Rev P	ot interpr	ited Heat	Fiel .	,													
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Sort by Bin	*		carotaGetlad	25.8	briding	1.00e-3	100e-6	No	Single	0.966	523e-9	-4.01	-9.57	-4.54	0	5.16-4	N/K	Ne.	N/sk	N/A	14/06	34/4	ACA.
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29. 除了上述二者,可以選擇 Signature plot 觀察反應自由能及其組成(Gibbs free energy ( $\Delta G$ ), enthalpy change ( $\Delta H$ ), and change in entropy ( $\Delta S$ ))

Presentation	Overview Adjust Baseline Assign Controls Adjust Fit Processor B 🔛 🔛 🐼	
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