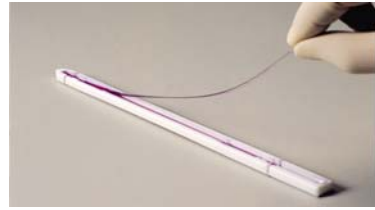


IPGphor 3 簡易操作流程

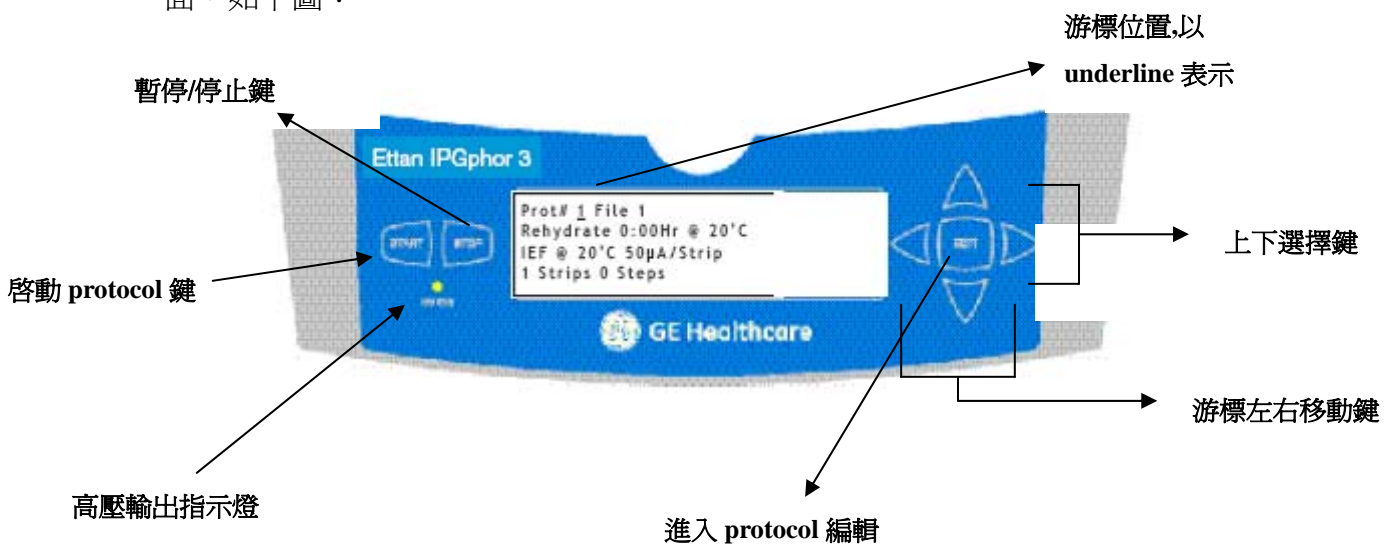
1. 依照所跑的不同 IEF strip 長度，pH 範圍及染色方法來選擇所需要的樣品量 (table 1)，並將樣品與覆水液 (rehydration buffer)做充分混合 (table 2)。
2. 將樣品及覆水液均勻的加在 strip holder，將 dry strip 的塑膠保護膜撕掉，小心的放到 strip holder 中 (膠面朝下)，避免有氣泡產生。



3. 確定沒有氣泡後再加入 cover fluid 覆蓋 dry strip,蓋上 strip holder 的蓋子。
4. 將 strip holder 小心的移至 IPGphor 上，放上 lid adapter 固定 holder 並蓋上機器的蓋子。



5. 啓動 IPGphor 的電源(位於左後方)，此時機器會自動檢測，約 30 秒後機器會進入主畫面，如下圖：



6. 利用面板上的上下鍵選擇所需的 protocol 編號，IPGphor 3 最多可以儲存 10 組 protocol，利用左右鍵移動游標至 File 的位置，利用上下鍵選擇數字及字母編輯 protocol 名稱。

```
Prot# 1 File 1
Rehydrate 0:00Hr @ 20°C
IEF @ 20°C 50uA/Strip
1 Strips 0 Steps
```

7. 利用左右鍵移動游標至 Rehydration 溫度與時間的設定位置，利用上下鍵輸入所需的條件。同樣地可利用左右鍵移動游標至 IEF 溫度及電流的設定位置，利用上下鍵輸入所需的條件。

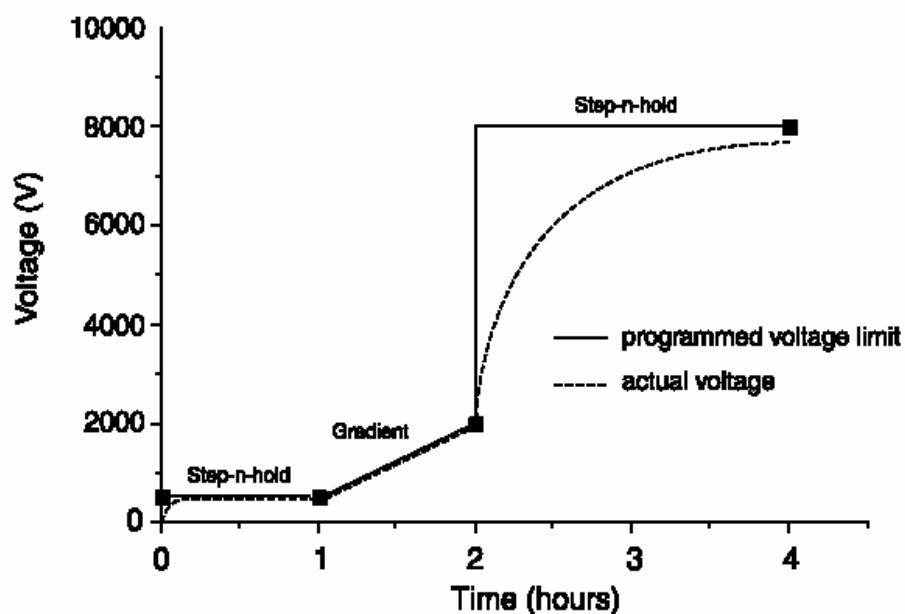
```
Prot# 1 File 1
Rehydrate 10:00Hr @ 20°C
IEF @ 20°C 50uA/Strip
1 Strips 0 Steps
```

```
Prot# 1 File 1 Rehydrate
10:00Hr @ 20°C
IEF @ 20°C 50uA/Strip
1 Strips 0 Steps
```

8. 按下 EDIT 鍵進入 IEF step 的設定，在每個 protocol 中共有 9 個步驟 S1-S9 可供設定，利用左右鍵移動游標至各種設定之下，再使用上下鍵去輸入所需的條件，各種設定分述如下：

```
Prot#1 3 Steps
S1 Grad 500V 1:00 Hrs ——— hours step
S2 Grad 1000V 4:00 Hrs
S3 Step 8000V 80000 Vhr ——— volt-hours step
```

9. 在每個步驟下有 step-n-hold 及 gradient 二種不同的模式可選擇，所代表的意義是電壓上升情況的選擇，如下圖：



利用左右鍵移動游標至 step 的 s，再利用上下鍵則可切換 Step 及 Grad 兩種不同的設定模式。

10. 利用左右鍵移動游標至電壓設定處，再利用上下鍵選擇所需的電壓。

11. 移動游標至時間的設定位置，除了可利用上下鍵輸入所需的時間外，也可以選擇利用 Vhrs 來設定所需要的條件，只要將游標移至 Hrs，則可利用上下鍵切換 Hrs 及 Vhrs 兩種不同的設定模式。

```
Prot#1 3 Steps
S1 Grad 500V 1:00 Hrs
S2 Grad 1000V 4:00 Hrs
S3 Step 8000V 80000 Vhr
```

12. 移動游標至 S1，利用上下鍵則可選擇 S1 到 S9，可依個人需要來設定。

13. 確定設定好所需的各種條件之後，按下 EDIT 鍵回到主畫面。

按下面板上的 START 鍵，此時會出現畫面如下：

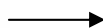
```
Number of strips :12
Press START to continue
```

```
Prot# 1 MY PROTOCOL
Rehydrate 10:00Hr @ 20°C
IEF @ 20°C 200uA/Strip
12 Strips 3 Steps
```

使用上下鍵輸入每次所跑的 strip 數目，最多可至 12 條。

14. 再按一次 START 鍵，則會出現畫面如下圖左，約幾秒之後，則進入 rehydration step，之後開始進行 IEF 的各個步驟

```
Starting protocol # 1
3 steps 4:00 Hr
```



```
Running Prot# 1
Rehydration for 10:00 Hr
0:01 Hr Elapsed
Rehydrate at 20°C
```

15. 在過程中若有需要機器暫停則按一次 STOP 鍵，此時機器會呈現暫停狀態，可按下 START 鍵繼續實驗，或再按一次 STOP，此時機器則會真正停止。

```
Paused in step 1
Press START to continue
```

```
Run ended at
8000V 12000 Vhrs Total
50uA 8:00 Hr Total
Press Stop to reset
```

16. 當機器跑完所設定的條件後，則會出現畫面如右上，此時可按下 **STOP** 鍵回到主畫面，將 Strip 用鑷子夾起，可放在-80°C 保存最多一周，或直接進行平衡步驟，開始跑 SDS-PAGE。
17. 此時可將機器直接關機。
18. 使用過的 strip holder 請用 2%-5%的 strip holder cleaning solution 清洗，並可選擇軟毛的牙刷刷洗 holder 的電極，清洗後用二次水沖洗乾淨，即可晾乾保存。

Table 1 Rehydration solution volume per Immobiline Drystrip (including sample)

IPG strip length (cm)	Total volume per strip* (µl)	Oil (µl)
7 cm	125 µl	200
11 cm	200 µl	400
13 cm	250 µl	500
18 cm	340 µl	800
24 cm	450 µl	1000

Table 2 Guidelines for running 7–24-cm Immobiline DryStrip gels on Ettan IPGphor 3 Isoelectric Focusing Unit. Running conditions: Temperature 20 °C; current 50 µA per strip except where noted.

7-cm strips

pH intervals	Voltage mode	Voltage (V)	Time (h:min)	kVh
3–11 NL	1 Step and Hold*	300	0:30*	0.2
3–10	2 Gradient	1000	0:30	0.3
6–11	3 Gradient	5000	1:20	4.0
	4 Step and Hold	5000	0:06–0:25	0.5–2.0
	Total		2:26–2:45	5.0–6.5
3–10 NL	1 Step and Hold*	300	0:30*	0.2
4–7	2 Gradient	1000	0:30	0.3
3–5.6 NL	3 Gradient	5000	1:30	4.5
	4 Step and Hold	5000	0:12–0:36	1.0–3.0
	Total		2:42–3:06	6.0–8.0
7–11 NL	1 Step and Hold*	300	0:30*	0.2
	2 Gradient	1000	1:00	0.7
	3 Gradient	5000	1:30	4.5
	4 Step and Hold	5000	0:20–0:55	1.6–4.6
	Total		3:20–3:55	7.0–10.0
5.3–6.5	1 Step and Hold*	300	1:00*	0.2
6.2–7.5	2 Gradient	1000	1:00	0.7
	3 Gradient	5000	2:30	7.5
	4 Step and Hold	5000	0:45–1:30	3.6–7.6
	Total		5:15–6:00	12.0–16.0

* When running crude samples, step 1 may be extended up to 4 h to allow salt to migrate out of the strip at low voltage.

11-cm strips

pH intervals	Step Voltage mode	Voltage (V)	Time (h:min)	kVh
3-11 NL	1 Step and Hold*	500	1:00	0.5
3-10	2 Gradient	1000	1:00	0.8
6-11	3 Gradient	6000	2:00	7.0
	4 Step and Hold	6000	0:10-0:40	0.7-3.7
	Total		4:05-4:40	9.0-12.0
4-7	1 Step and Hold*	500	1:00	0.5
3-5.6 NL	2 Gradient	1000	1:00	0.8
	3 Gradient	6000	2:30	8.8
	4 Step and Hold	6000	0:10-0:50	0.9-4.9
	Total		4:40-5:20	11.0-15.0
7-11 NL	1 Step and Hold*	500	1:00	0.5
	2 Gradient	1000	1:00	0.8
	3 Gradient	6000	2:30	8.8
	4 Step and Hold	6000	0:50-1:40	4.9-9.9
	Total		5:20-6:10	15.0-20.0
5.3-6.5	1 Step and Hold†	500	1:00†	0.5
6.2-7.5	2 Gradient	1000	1:00	0.8
	3 Gradient	6000	3:00	10.5
	4 Step and Hold	6000	2:40-3:50	16.2-23.2
	Total		7:40-8:50	28.0-35.0

* When running crude samples, step 1 may be extended up to 4 h to allow salt to migrate out of the strip at low voltage.

† To convert this to a convenient overnight run, extend Step 1 to 6 h (3 kVh) and reduce step 4 by 3 kVh.

13-cm strips

pH intervals	Step Voltage mode	Voltage (V)	Time (h:min)	kVh
3-10	1 Step and Hold*	500	1:00	0.5
3-11 NL	2 Gradient	1000	1:00	0.8
6-11	3 Gradient	8000	2:30	11.3
	4 Step and Hold	8000	0:10-0:30	1.4-4.4
	Total		4:40-5:00	14.0-17.0
3-10 NL	1 Step and Hold*	500	1:00	0.5
4-7	2 Gradient	1000	1:00	0.8
3-5.6 NL	3 Gradient	8000	2:30	11.3
	4 Step and Hold	8000	0:25-0:55	3.4-7.4
	Total		4:55-5:25	16.0-20.0
7-11 NL	1 Step and Hold*	500	1:00	0.5
	2 Gradient	1000	1:00	0.8
	3 Gradient	8000	3:00	13.5
	4 Step and Hold	8000	0:45-1:15	6.2-10.2
	Total		5:45-6:15	21.0-25.0
5.3-6.5	1 Step and Hold†	500	1:00†	0.5
6.2-7.5	2 Gradient	1000	1:00	0.8
	3 Gradient	8000	3:00	13.5
	4 Step and Hold	8000	2:55-4:10	23.2-33.2
	Total		7:55-9:10†	38.0-48.0

* When running crude samples, step 1 may be extended up to 4 h to allow salt to migrate out of the strip at low voltage.

† To convert this to a convenient overnight run, extend Step 1 to 6 h (3 kVh) and reduce step 4 by 3 kVh.

18-cm strips

Note: When using IPGphor Manifold and 10 kV, set current limit to 75 μ A per strip and follow step 1, 2, 3b and 4b. Using IPGphor Regular Strip Holder or Cup Loading Strip Holder with the 18- and 24-cm strips, the maximum allowed voltage is 8000 V and current 50 μ A per strip. Follow step: 1, 2, 3a, 4a.

pH intervals	Step Voltage mode	Voltage (V)	Time (h:min)	Volt-hours kWh
3-10	1 Step and Hold	500	1:00	0.5
3-11 NL	2 Gradient*	1000	1:00 (8:00)*	0.8 (6.0)
6-11	3a Gradient	8000	3:00	13.5
	4a Step and Hold	8000	0:46-1:30	6.2-12.2
	3b Gradient	10000	3:00	16.5
	4b Step and Hold	10000	0:20-0:55	3.2-9.2
	Total			21.0-27.0
3-10NL	1 Step and Hold	500	1:00	0.5
4-7	2 Gradient*	1000	1:00 (8:00)*	0.8 (6.0)
3-5.6 NL	3a Gradient	8000	3:00	13.5
	4a Step and Hold	8000	1:30-2:40	12.2-21.2
	3b Gradient	10000	3:00	16.5
	4b Step and Hold	10000	0:55-1:50	9.2-18.2
	Total			27.0-36.0
6-9	1 Step and Hold	500	1:00	0.5
7-11 NL	2 Gradient*	1000	1:00 (8:00)*	0.8 (6.0)
	3a Gradient	8000	3:00	13.5
	4a Step and Hold	8000	3:10-4:30	25.2-35.2
	3b Gradient	10000	3:00	16.5
	4b Step and Hold	10000	2:15-3:15	22.2-32.2
	Total			40.0-50.0
3.5-4.5	1 Step and Hold	500	1:00	0.5
4.0-5.0	2 Gradient*	1000	1:00 (6:00)*	0.8 (4.5)
4.5-5.5	3a Gradient	8000	3:00	13.5
5.0-6.0	4a Step and Hold	8000	4:55-5:40	39.2-45.2
5.5-6.7	3b Gradient	10000	3:00	16.5
	4b Step and Hold	10000	3:40-4:15	36.2-42.2
	Total			54.0-60.0
5.3-6.5	1 Step and Hold	500	2:00	1.0
6.2-7.5	2 Gradient*	1000	2:00 (3:00)*	1.5 (2.2)
	3a Gradient	8000	3:00	13.5
	4a Step and Hold	8000	6:45-8:40	54-69
	3b Gradient	10000	3:00	16.5
	4b Step and Hold	10000	5:05-6:35	51-66
	Total			70.0-85.0

* When using the IPGphor Cup Loading Strip Holder, running crude samples, or when a more convenient overnight run of 15-17 h is desired, the time in step 2 can be prolonged up to 8 h with a duration of 6.0 kWh. Using this option, step 4 can be reduced by the added kWh in step 2, to reach the specified total kWh.

24-cm strips

Note: When using IPGphor Manifold and 10 kV, set current limit to 75 μ A per strip and follow step 1, 2, 3b and 4b. Using IPGphor Regular Strip Holder or Cup Loading Strip Holder with the 18- and 24-cm strips, the maximum allowed voltage is 8000 V and current 50 μ A per strip. Follow step: 1, 2, 3a, 4a.

pH intervals	Step Voltage mode	Voltage (V)	Time (h:min)	kVh
3-11 NL	1 Step and Hold	500	1:00	0.5
3-10	2 Gradient*	1000	1:00 (8:00)*	0.8 (6.0)*
	3a Gradient	8000	3:00	13.5
	4a Step and Hold	8000	2:30-3:45	20-30
	3b Gradient	10000	3:00	16.5
	4b Step and Hold	10000	1:45-2:45	17.2-27.2
	Total			35-45
3-10 NL	1 Step and Hold	500	1:00	0.5
3-7 NL	2 Gradient*	1000	1:00 (7:00)*	0.8 (5.2)*
4-7	3a Gradient	8000	3:00	13.5
3-5.6 NL	4a Step and Hold	8000	3:45-5:36	30-45
	3b Gradient	10000	3:00	16.5
	4b Step and Hold	10000	2:45-4:15	27.2-42.2
	Total			45-60
6-9	1 Step and Hold	500	1:00	0.5
7-11 NL	2 Gradient*	1000	1:00 (5:00)*	0.8 (3.8)*
	3a Gradient	8000	3:00	13.5
	4a Step and Hold	8000	5:36-8:45	45-70
	3b Gradient	10000	3:00	16.5
	4b Step and Hold	10000	4:15-6:45	42.2-67.2
	Total			60-85
3.5-4.5	1 Step and Hold	500	2:00	1.0
4.0-5.0	2 Gradient*	1000	2:00 (5:00)*	1.5 (3.8)*
4.5-5.5	3a Gradient	8000	3:00	13.5
5.0-6.0	4a Step and Hold	8000	9:10-10:30	74-84
5.5-6.7	3b Gradient	10000	3:00	16.5
	4b Step and Hold	10000	7:05-8:05	71-81
	Total			90.0-100
5.3-6.5	1 Step and Hold	500	2:00	1.0
6.2-7.5	2 Gradient*	1000	2:00 (5:00)*	1.5 (3.8)*
	3a Gradient	8000	3:00	13.5
	4a Step and Hold	8000	11:45-14:15	94-114
	3b Gradient	10000	3:00	16.5
	4b Step and Hold	10000	9:05-11:05	91-111
	Total			110-130

* When using the IPGphor Cup Loading Strip Holder, running crude samples, or simply to adapt the run time to a convenient overnight run of 15-17 h, the time in step 2 can be prolonged by up to 8 h with a duration of 5.2 kVh. Using this option, step 4 can be reduced by the added kVh in step 2, to reach the specified total kVh.

IPGphor 3 簡易中文操作流程

