Two-Dimensional Electrophoresis - "*Large*" Gels

Always wear powder free disposable gloves.

Important: Only use the gelcompany buffer kit for the running buffers and equilibration solutions.

1. Apply 80 mL (2 x 40 mL) of each electrode buffer to the respective stack of four electrode wicks in the compartments of the gelcompany PaperPool (fig. 1) and soak for a minimum of 15 minutes.

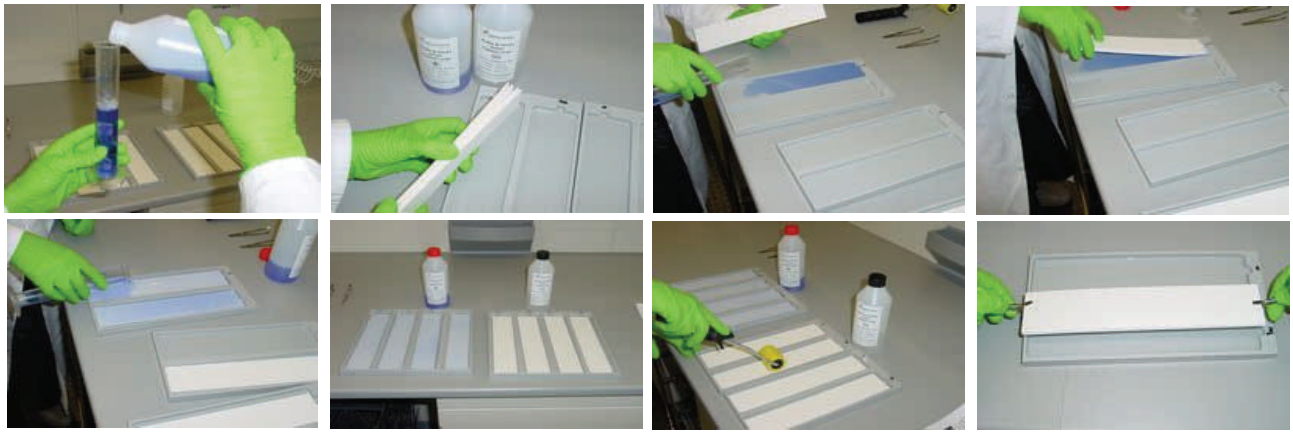


Fig. 1: Soaking stacks of four paper wicks in 80 mL electrode buffer. First pour 40 mL buffer into the empty PaperPool compartment, then lay the stack of paper wicks into the PaperPool and add another 40 mL buffer.

2. Prepare the two equilibration solutions from the gelcompany IPG-Strip equilibration buffer (Eq. buffer):

3. Equilibrate each IPG-strip (gel-side up) in 6 mL solution in a gelcompany equilibrator (cat# 1003-04; fig. 2) on an orbital shaker (30 rev/min).

***DTT solution**:* Weigh urea and dithiothreitol (DTT), and add the equilibration buffer according to table 1 and dissolve completely.
***IAA solution**:* Weigh urea and iodoacetamide (IAA), and add the equilibration buffer according the table 1 and dissolve completely.

Table 5.1. Preparing the equilibration solutions for 24 cm IPG strips:

Number of strips	Urea [g]	DTT [mg]	IAA [mg]	Eq. Buffer [mL]	Total volume [mL]
1	1.8	50	-	5	6
	1.8	-	125	5	6
2	3.6	100	-	10	12
	3.6	-	250	10	12
3	5.4	150	-	15	18
	5.4	-	375	15	18
4	7.2	200	-	20	24
	7.2	-	500	20	24

Step 1	in DTT solution	for 15 min
Step 2	in IAA solution	for 15 min

After the 2nd equilibration step discard the solutions.

NB: It is important not to use standard paper wicks for running gels if you are silver staining.

Please contact support@gelcompany.com for silver staining compatible electrode wicks.

For more detailed information on running gelcompany flatbed gels please visit our website where you can download a video and the HPE FlatTop manual which describe in more detail the running of these gels.

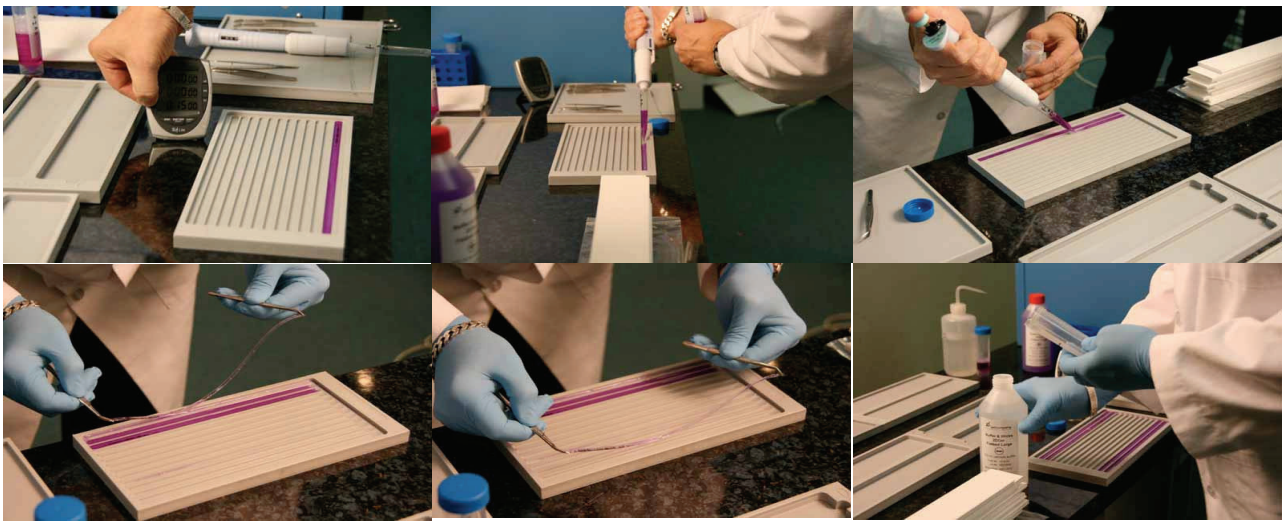


Fig. 2: Equilibration of the IPG-strips in 6 mL equilibration buffer.

4. Switch the thermostatic circulator on, set it to 15°C. Switch the FlatTop Tower on, and set the valve to "*Bypass*" to avoid water condensation on the gel surfaces.

5. Apply at least 4mL of cooling contact fluid onto the center of the cooling plate (fig. 3)

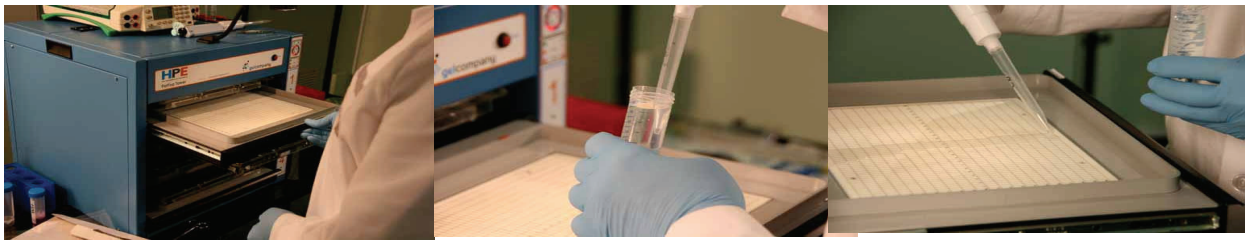


Fig. 3: Application of the cooling fluid

6. Grip the gel (surface up) at the two lateral edges at the protruding film, bend into a "*U-shape*" and slide the film-backing left and right on the cooling plate to distribute the cool contact fluid evenly (Fig. 4).

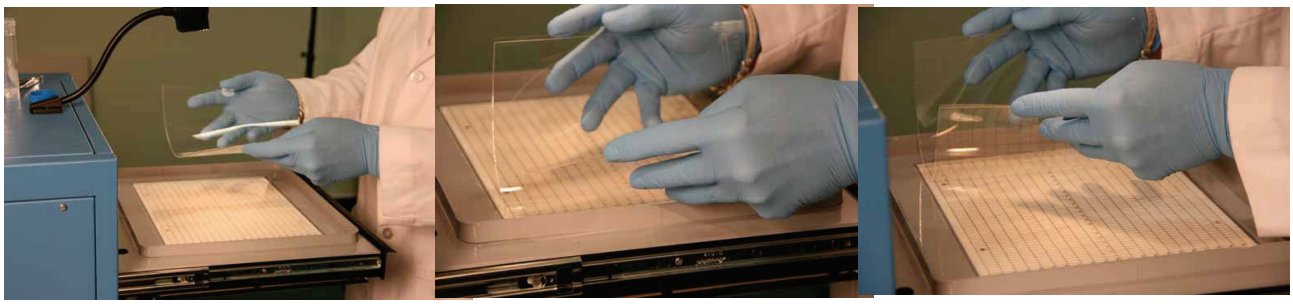


Fig. 4: Placing the gel on the cooling plate.

7. Remove excess cooling fluid along the film edges with lint-free paper tissue.

8. Finally lower the the gel onto the cooling plate: the IPG strip-slot towards the cathode, the cathodal edge of the IPG strip-slot matching line "*19*" (fig. 5).

Fig. 5: Placing Flatbed *Large* gel on the cooling plate.

9. Remove excess electrode buffer from the wicks by tilting the electrode stack along one long edge and dab it on the PaperPool bottom (fig. 6). Always hold the wicks horizontally, do not tilt them, this would cause a higher buffer concentration on one side.

Fig. 6: Removal of excess buffer from the wicks. Always hold the wicks horizontally.

10. Place the stack of electrode wicks onto the gel edges, overlapping the gel by at least 2 mm. Smooth out air bubbles using a roller. Move the anodal wicks around the gel area to avoid dropping buffer on the gel surface (fig. 7)

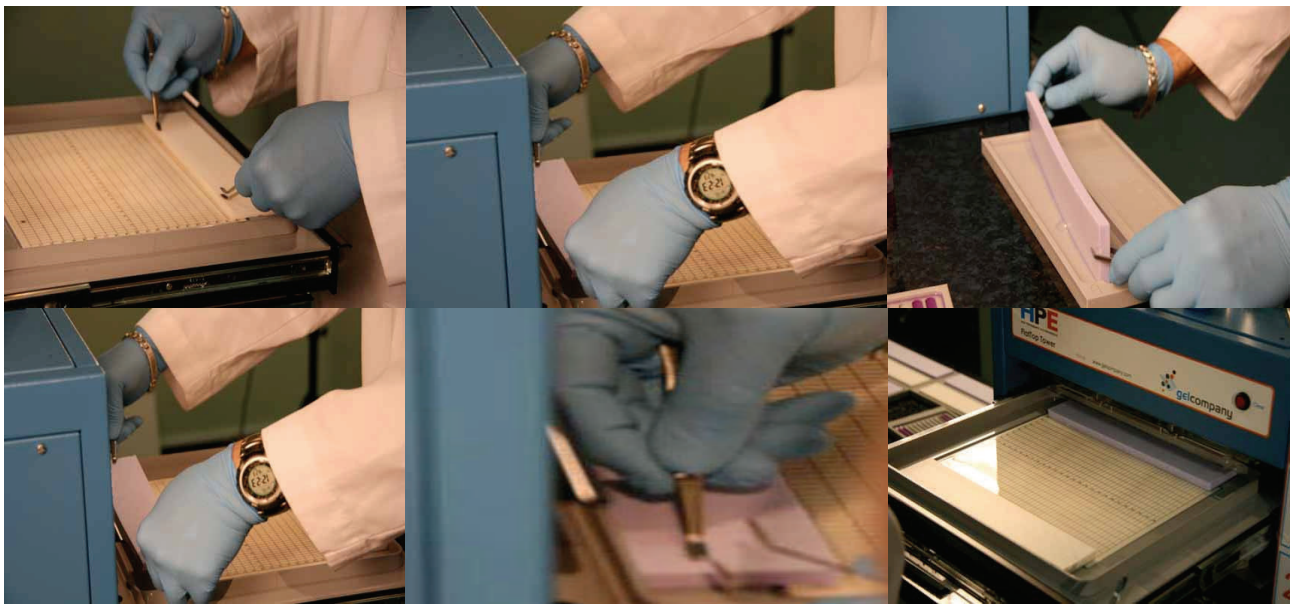
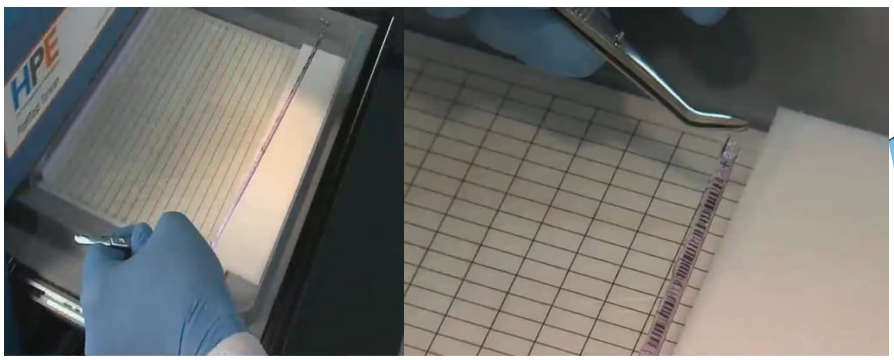


Fig. 7: Applying the stack of electrode wicks on gel edges.

11. Trim the film support of the IPG-strips on both sides.

12. Place the IPG strip gel-side down anodal side to the right, into the slot of the Flatbed Gel (fig. 8). Start in the middle to prevent accumulation of buffer towards one side. Push it carefully towards the anode edge of the slot. Slide along the backing of the strip with the forceps to ensure good contact to the bottom of the slot.



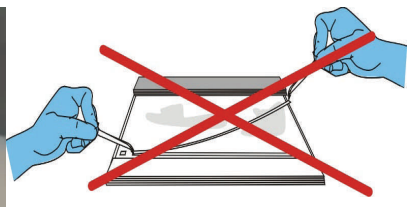


Fig. 8: Application of the IPG strip into the slot of the 2DGel flatbed. Always hold the strip horizontally.

13. Apply 5 µL SDS marker proteins to the marker well.

14. Close the lid while lowering the electrodes on the wicks, plug in the electrode connectors, turn the valve to "*cooling*" (15 °C), switch on the FlatTop Tower main switch (pumps and electronic control) and start the run according to tables 2 or 3 (*see below*.)

15. After 1 hour 10 min interrupt the run by standby/pausing/freezing the power supply, open the drawers and remove the IPG-strip(s) and continue the run.

Tab. 5.2: Running conditions (day run), cooling temperature 15 °C

Steps	Voltage	Current				Power				Time
		1 gel	***2 gels***	***3 gels***	***4 gels***	***1 gel***	***2 gels***	***3 gels***	***4 gels***	
S1	100 V †	7mA ††	14mA ††	21mA ††	28 mA ††	1 W ††	2 W ††	3 W ††	4 W ††	30 min
S2	200 V †	13 mA ††	26 mA ††	39 mA ††	52 mA ††	3 W ††	6 W ††	9 W ††	12 W ††	30 min
S3	300 V ††	20 mA †	40 mA †	60 mA †	80 mA †	5 W ††	10 W ††	15 W ††	20 W ††	10 min
after this step: **remove the IPG strip.**										
S4	1500 V ††	40 mA †	80 mA †	120 mA †	160 mA †	30 W ††	60 W ††	90 W ††	120 W ††	3h 50min*

* valid for homogeneous gels, for the gradient gel 10-15 this step 4 takes **4 h 50 min**.

Tab. 5.3: Running conditions over night for homogeneous gels**, cooling temperature 15 °C

Steps	Voltage	Current				Power				Time
		1 gel	***2 gels***	***3 gels***	***4 gels***	***1 gel***	***2 gels***	***3 gels***	***4 gels***	
S1	100 V †	7mA ††	14mA ††	21mA ††	28 mA ††	1 W ††	2 W ††	3 W ††	4 W ††	30 min
S2	200 V †	13 mA ††	26 mA ††	39 mA ††	52 mA ††	3 W ††	6 W ††	9 W ††	12 W ††	30 min
S3	300 V ††	20 mA †	40 mA †	60 mA †	80 mA †	5 W ††	10 W ††	15 W ††	20 W ††	10 min
after this step: **remove the IPG strip.**										
S4	220 V ††	5 mA †	10 mA †	15 mA †	20 mA †	3 W ††	6 W ††	9 W ††	12 W ††	15 hours

**** Please note:** It is **not** recommended to run the gradient gel 2DGel flatbed 10-15 overnight.

For programming BioRad Power Supplies only:

† - Set as "*constant*"

†† - Set as "*limit*"

Legal Information

Gelcompany Pty Limited,
Unit 3 43 – 51 College Street,
. Gladesville, NSW 2111, Australia.

Ordering Information

		Quantity	*gelcompany code no.*
Equipment:			
HPE-FlatTop Tower		1	PPT-001-TI
HPE-Thermostatic Circulator		1	PPC-100-TI
HPE-Power supply		1	PPP-001-TI
Accessories:			
HPE-FlatTop Tower Accessory Kit*			
*Comprising, 4 PaperPools, Scalpel handle, pack of scalpel blades, 2 forceps pointed, 2 forceps blunt, 4 Falcon tubes, scissors and roller		1 kit	PGT-001-TK
PaperPool (Tray for soaking the electrode strips)		4	1003-03
IPG Strip Equilibrator (Tray for IPG strip rehydration and equilibration)		1	1003-04
Steel Tray Standard (with grid, for hot Coomassie staining)		1	1003-25
Steel Tray Large (for large gels, with grid, for hot Coomassie staining)		1	1003-26
Steel Tray Multi 6 (for up to 6 large gels, with 6 grids)		1	1003-27
ScanFrame (for standard and large size gels)		1	PAS-001-TI
Cooling fluid			
HPE-FlatTop Cooling Fluid	50 mL	1	PCC-050-TI
	150 mL	1	PCC-150-TI

Gels and buffer kits (examples only - for other gel types please enquire)

1D SDS Electrophoresis - Standard size (25 x 12.5 cm) gels

Ready to use kits for 1D SDS-electrophoresis including cooling fluid, buffers and electrode wicks

10%*T*, 25 slots for 15µL (4 gels)	1 pack	PCS-125-TK
10%*T*, 52 slots for 6µL (4 gels)	1 pack	PCS-252-TK
15%*T*, 25 slots for 15µL (4 gels)	1 pack	PCS-325-TK
15% *T*, 52 slots for 6µL (4 gels)	1 pack	PCS-452-TK
12.5% *T*, 25 slots for 15µL on non-fluorescent film-backing* (4 gels)	1 pack	PCS-425-TK

*for DIGE-samples and other fluorescent staining

2D Electrophoresis Standard Size (25 x 12.5 cm) gels

HPE-2DGel flatbed NF Kits "Double gels":

Ready to use kits for running 2 X 11cm IPG-strips (*Double Gels*) including cooling fluid, buffers and electrode wicks.

2DGel flatbed NF 12.5%, 4 gels	1 pack	PCF-902-TK
2DGel flatbed NF 12.5%, 20 gels	1 pack	PCF-202-TK
2DGel flatbed NF 10-15% (gradient gels) 4 gels	1 pack	PCF-903-TK
2DGel flatbed NF 10-15% (gradient gels) 20 gels	1 pack	PCF-203-TK

HPE-2DGel flatbed NF Kits "Triple gels":

Kits for running 3 X 7 cm IPG-strips (*Triple Gels*) including cooling fluid, buffers, wicks and electrodes.

2DGel flatbed NF 12.5%, 4 gels	1 pack	PCT-301-TK
2DGel flatbed NF 12.5%, 20 gels	1 pack	PCT-302-TK
2DGel flatbed NF 10-15% (gradient gels) 4 gels	1 pack	PCT-321-TK
2DGel flatbed NF 10-15% (gradient gels) 20 gels	1 pack	PCT-322-TK

HPE-*Large* Format 2D Gels 25 X 19.5cm

Kits for running 1 X 24cm IPG-strips (Large Gels) including cooling fluid, buffers and electrode wicks.

2DGel flatbed NF 12.5%, 4 gels	1 pack	PCF-906-TK
2DGel flatbed NF 12.5%, 20 gels	1 pack	PCF-206-TK
2DGel flatbed NF 10-15% (gradient gels) 4 gels	1 pack	PCF-907-TK
2DGel flatbed NF 10-15% (gradient gels) 20 gels	1 pack	PCF-207-TK

Additives:

IPG-Ampholyte Mix, 1mL (40% w/v) for rehydration of IPG-strips.	1 pack	1004-15

Additionally Required (use only highest quality):

Urea, Dithiothreitol (DTT), Iodoacetamide (IAA), SDS marker proteins.

Fluorescent Stain Kit

Kit includes all fixatives and buffers required for fluorescently staining gels:

LavaPurple Protein Kit 100 mL	1 pack	LP-011100
LavaPurple Protein Kit 25 mL	1 pack	LP-011025

This protocol replaces DIN 1010-02-09

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