Two-Dimensional Electrophoresis - "*Double*" and "*Triple*" Gels

Always wear powder free disposable gloves.

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

1. Prepare the two equilibration solutions from the gelcompany IPG Strip equilibration buffer (Eq. buffer):
   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 1. Preparing the equilibration solutions for 11 and 7 cm IPG strips: 2.

Number X Size of strips	Urea [g]	DTT [mg]	IAA [mg]	Eq. Buffer [mL]	Total volume [mL]
2X11cm or 3X7cm	1.8	50	-	5	6
	1.8	-	125	5	6
4X11cm or 6X7cm	3.6	100	-	10	12
	3.6	-	250	10	12
6X11cm or 9X7cm	5.4	150	-	15	18
	5.4	-	375	15	18
8X11cm or 12X7cm	7.2	200	-	20	24
	7.2	-	500	20	24

2. Equilibrate each strip (gel-side up) in 3 mL (11 cm strips) or 2 mL (7 cm strips) solution in an equilibrator (fig. 1) on an orbital shaker at 30 rev/min:

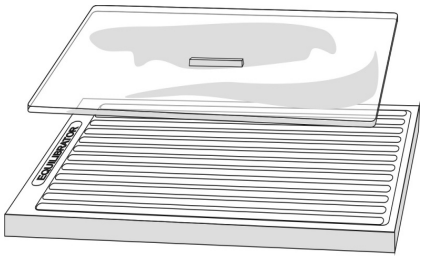


Fig. 1

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

3. After the 2nd equilibration discard the solution.

4. Apply 20 mL of each electrode buffer to the respective electrode wick in the compartments of the gelcompany PaperPool (fig. 2).

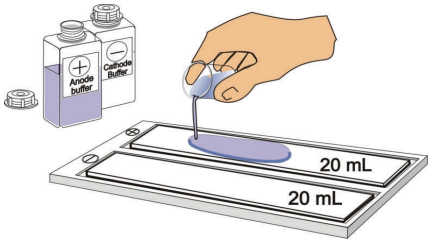
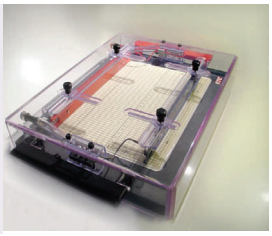


Fig. 2

5. Switch the thermostatic circulator on and set to 15°C. If available (e.g. on HPE FlatTop Tower or FlatTop) set the bypass valve to "*By-pass*" to avoid water condensation on the gel surface.

HPE FlatTop Tower



FlatTop Large

6. Apply at least 3 mL of cooling contact fluid onto the cooling plate to ensure good cooling contact.

7. 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).

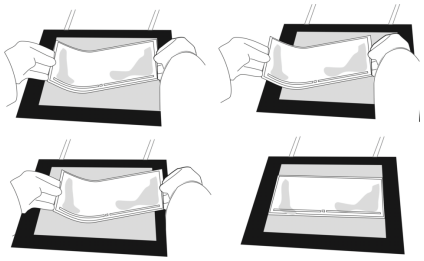


Fig. 4

8. Place the gel onto the cooling plate: the IPG strip-slot towards the cathode, the cathodal edge of the IPG strip-slot matching line “15“ (fig 5).

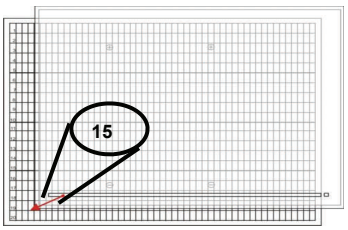


Fig. 5

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

10. Remove excess electrode buffer from the wicks by tilting the electrode wicks along one long edge and dab it on the PaperPool bottom (fig. 6).

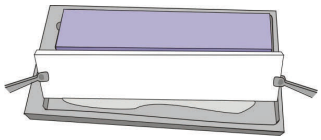


Fig.6

11. Place the electrode wicks onto the gel edges overlapping them by at least 2 mm. Hold wicks horizontally, never at an angle as this causes unequal buffer concentration along the wick.

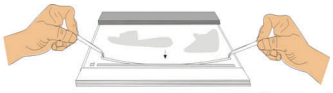


Fig. 7

12. Trim the film support of the IPG strips on both sides. Place the IPG strips gel-side down, anodal sides to the right, into the slots of the double or triple gels and push them carefully towards the anode edges of the slots (fig. 8). Gently slide along the backing of the strips with the forceps to ensure good contact to the bottom of the slots.

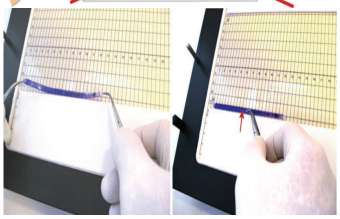


Fig. 8

13. Apply 5 µL SDS marker proteins to the marker well(s).

14. Close the lid while lowering the electrodes on the wicks, plug in the cables, switch the bypass valve to “*cooling*“ (15°C),and start the run according to table 2.

15. After 1 hour 10 min interrupt the run, remove the IPG strip(s), and then continue the run.

Running conditions: See table .2 for the maximum settings.

Table. .2: Running conditions (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 ††	26mA ††	39 mA ††	52 mA ††	3 W ††	6 W ††	9 W ††	12 W ††	30 min
S3	300 V ††	20mA †	40 mA †	60 mA †	80 mA †	5W ††	10 W ††	15 W ††	20 W ††	10 min
after this step: **remove the IPG strips.**										
S4*	1000 V ††	40 mA †	80 mA †	120 mA †	160 mA †	25 W ††	50 W ††	75 W ††	100 W ††	2h*

* valid for homogeneous gels, for the gradient gel 10 -15% this step 4 takes **2.5 h** .

For programming BioRad Power Supplies only:

† - Set as “*constant*“

†† - Set as “*limit*“

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 HPE FlatTop manual which describe running of these gels in more detail .

Legal Information

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Related Products

		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 1009-02-09

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