Instruction Manual: 2D Electrophoresis with 2D HPE™ Double and Triple Gels

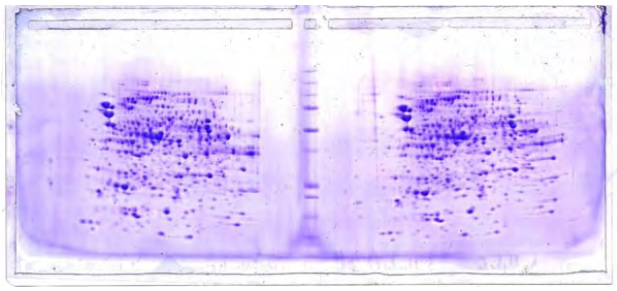

Fig. 1a: Double gel: 2x11 cm IPG strips, Coomassie staining

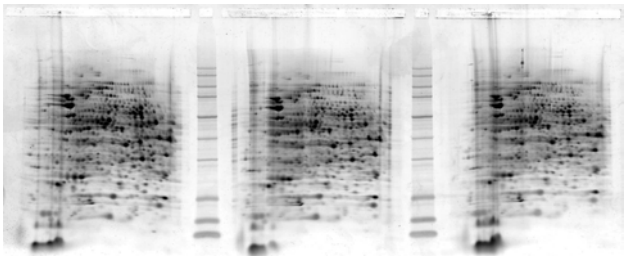

Fig. 1a: Triple gel: 3 x 7 cm IPG strips, LavaPurple staining

Always wear powder free disposable gloves.

Important: Only use the SERVA buffer kit for the running buffers and equilibration solutions!

1. Prepare the two equilibration solutions from the IPG Strip equilibration 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 them completely.

Tab. 1. Preparing the equilibration buffers for 11 and 7 cm IPG strips

Number of strips	Urea [g]	DTT [mg]	IAA [mg]	E. Buffer [ml]	Total volume [ml]
2x11 cm or 3x7 cm	1.8	50	-	5	6
	1.8	-	125	5	6
4x11 cm or 6x7 cm	3.6	100	-	10	12
	3.6	-	250	10	12
6x11 cm or 9x7 cm	5.4	150	-	15	18
	5.4	-	375	15	18
8x11 cm or 12x7 cm	7.2	200	-	20	24
	7.2	-	500	20	24

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

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

Fig. 2

3. After the 2nd equilibration discard the solution.

4. Apply 45 ml of each electrode buffer to the respective electrode wick in the compartments of the PaperPool (fig. 3).

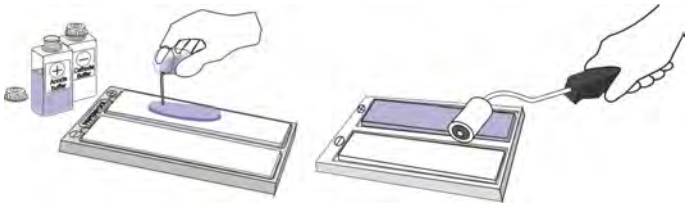

Fig. 3

5. Apply 3 mL of cooling contact fluid onto the cooling plate for good cooling contact.

6. Switch on the thermostatic circulator to 15 °C, and set the tubing or bypass valve to „by-pass“ to avoid water condensation on the gel surface.

7. Grip the gel (surface up) at the two lateral edges at the protruding film, bend it like an „U“ and slide the film-backing left and right on the cooling plate to distribute the cool contact fluid evenly (Fig. 4)

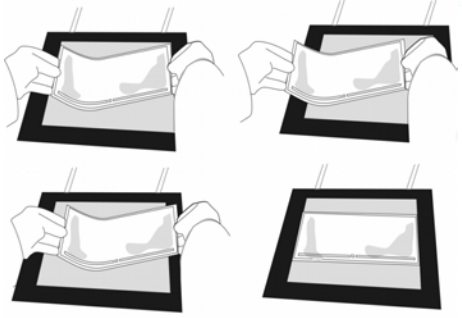

Fig. 4

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

9. Remove excess electrode buffer from the wicks by tilting the electrode wicks along one long edge and dab it on the PaperPool bottom (fig. 5).

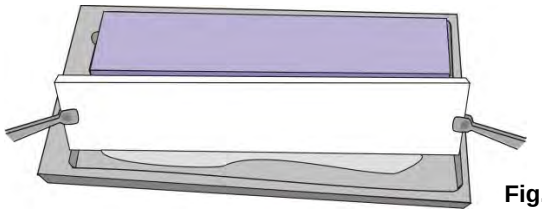

Fig. 5

8. Place the electrode wicks onto the gel edges overlapping them by at least 2 mm. Hold wicks horizontally! Never sloped, because this would cause unequal buffer concentration along the wick. Smooth out air bubbles with bent tip forceps.

9. 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 SDSGel and push them carefully towards the anode edges of the slots (fig. 6). Slide along the backing of the strips with the forceps to ensure good contact to the bottom of the slots.

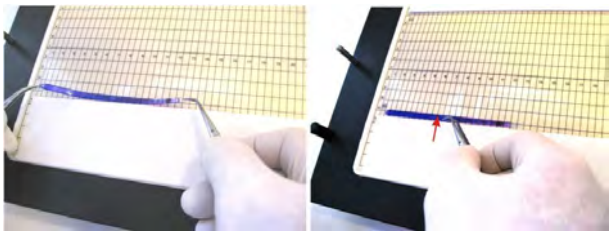

Fig. 6

10. Apply 5 µl SDS marker proteins to the well(s).

11. Close the lid, lower the electrodes on the wicks, turn the valve to cooling (15 °C), and start the run according to table 2.

12. After 1 h 10 min interrupt the run (press „pause“ or „wait“ on the power supply), remove the IPG strips and then continue the run.

Tab. 2: Running conditions for 1 gel (15 °C)

1 Gel:	Limit V	Limit mA	**Set W**	**Time**
phase 1	100 V	7 mA	1 W	30 min
phase 2	200 V	13 mA	3 W	30 min
phase 3	300 V	20 mA	5 W	10 min
after this step: **remove the IPG strip!**				
phase 4	1000 V	40 mA	25 W	2 h *)

*) valid for homogeneous gels, for the gradient gel 10—15 % this step 4 takes 2 h 30 min.

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