SERVA Electrophoresis

Instruction Manual: 2D Electrophoresis with 2D HPE™ Large Gels

Always:

- Wear powder-free disposable gloves.
- Do not touch or contaminate the gel surface.
- Only handle gel by the film margins.
- Store gels in a refrigerator but do not freeze.
- Only use SERVA buffer kit and equilibration solutions.

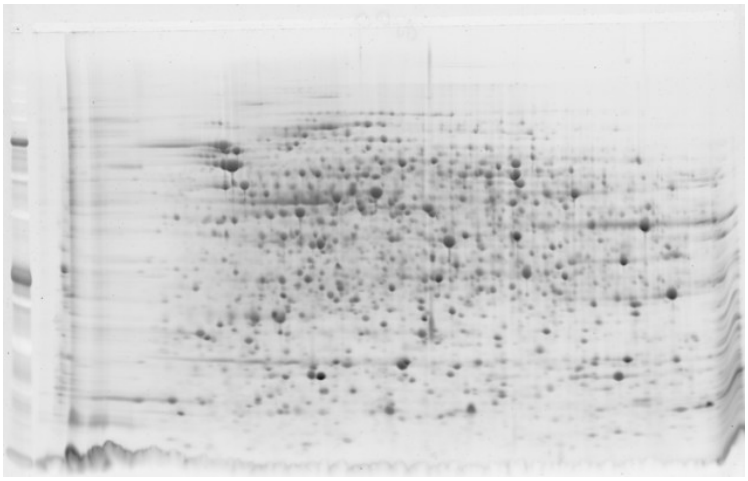

Fig. 1: 2DGel Flatbed Large, 24 cm IPG strip, LavaPurple Staining

1. Apply 45 ml of each electrode buffer to the electrode wicks in the compartments of the Paper-Pool (fig. 2), distribute the solution evenly with a roller,

2. Prepare the two equilibration solutions from the SERVA 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.

3. Equilibrate the IPG strip in 6 mL solution in an equilib-

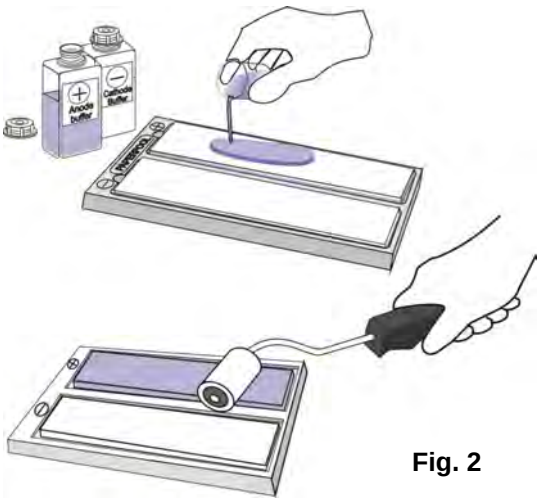

Fig. 2

Tab 1. Preparing the equilibration buffers for 24 cm IPG strips:

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

rator (fig. 3) on an orbital shaker with 30 rev/min:

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

After the 2nd equilibration discard the solution.

Fig. 3

4. Switch on the thermostatic circulator to 15 °C, and set the valve to „by-pass“ to avoid water condensation on the gel surfaces.

5. Apply 4.5 ml of cooling contact fluid onto the cooling plate.

6. 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). Place the gel onto the cooling plate: the IPG strip-slot towards the cathode, the cathodal edge of the IPG strip-slot matching line „18.5“ on the HPE Tower cooling plate, or 15 on the FlatTop Large.

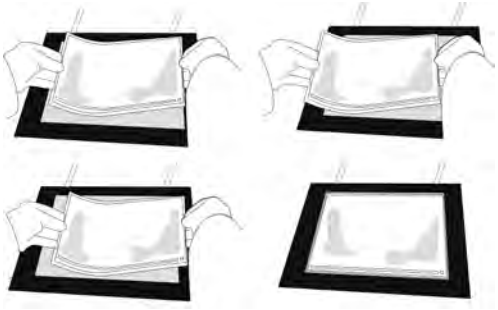

Fig. 4

7. Remove excess electrode buffer from wicks by tilting the electrode stack along one long edge and dab it on the paper pool bottom (fig. 5).

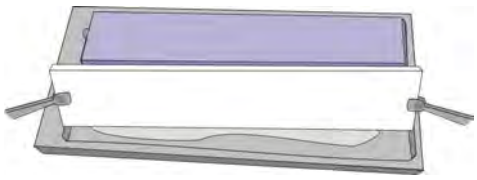

Fig. 5

8. Place the electrode wicks onto the gel edges. Overlapping them by at least 2 mm. Smooth out air bubbles with bent tip forceps.

9. Trim the film support of the strips on both sides.

10. Place the IPG strip gel-side down (!), anodal side to the right, into the slot of the SDSGel (fig. 6). Start in the middle, this prevents 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.

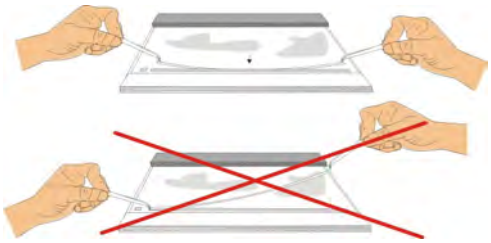

Fig. 6

12. Close the lid while lowering the electrodes on the wicks, turn the valve to cooling (15 °C), switch on the FlatTop Tower main switch (pumps and electronic control), and start the run acc. to tables 2 or 3

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

Running conditions for a homogeneous gel 12.5 % *T*:

Tab. 2: Running conditions at 15 °C **Short run:**

Phase	max V	max mA	max W	Time
1	100 V	7 mA	1 W	30 min
2	200 V	13 mA	3 W	30 min
3	300 V	20 mA	5 W	10 min
after this step: **remove the IPG strip!**				
4	1000 V	40 mA	30 W	3 h 50 min*)
5	1500 V	45 mA	40 W	40 min

Tab. 3: Running conditions at 15 °C **Overnight run:**

Phase	max V	max mA	max W	Time
1	100 V	7 mA	1 W	30 min
2	200 V	13 mA	3 W	30 min
3	300 V	20 mA	5 W	10 min
after this step: **remove the IPG strip!**				
4	220 V	5 mA	2 W	Hours until 6:00 a.m. next day
5	1000 V	40 mA	30 W	3 h*)

*)valid for homogeneous gels, for the **gradient gel 10-15** this phase 4 takes 4 h 30 min.

Important hint for staining: Fixing of film-backed gels is efficient with 15 % ethanol / 1 % citric acid, because the film-bound gels cannot become larger. Avoid solutions with alcohol concentrations above 20 % (v/v) to prevent gels coming off the film.

SERVA Electrophoresis GmbH, Carl-Benz-Str. 7, D-69115 Heidelberg, Germany.
Phone +49 6221 13840-0, Fax +49 6221 13840-10; E-Mail: info@serva.de
www.serva.de