Equilibration and loading of IPG-strips on 2DGel Flatbed Large

Always:

- Wear powder free disposable gloves.
- Store gels in a refrigerator but do not freeze.
- Only use **gel**company buffer kit and equilibration solutions.

1. Apply 40 mL of cathode electrode buffer to the a stack of 4 electrode wicks in the PaperPool. Repeat the operation using the anode buffer using a separate stack of 4 wicks (fig. 1). Leave the wicks for at least 10 mins so that they fully absorb the buffer.

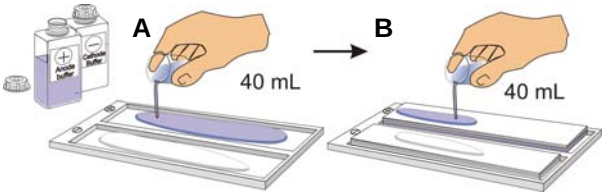

Fig. 1

2. Prepare two equilibration solutions in **gel**company IPG-strip equilibration buffer:
   DTT solution: Weigh urea and dithiothreitol (DTT), and add the equilibration buffer according to table 1 *(overleaf)* and dissolve completely.
   IAA solution: Weigh urea and iodoacetamide (IAA), and add the equilibration buffer according the table 1 and dissolve completely.
3. Using **gel**company equilibrator (fig 2. cat # 1003-04) on an orbital shaker (30 rev/min) equilibrate each strip in DDT solution (6 mL) for 15 min. Then move the strip to a slot containing IAA solution (6 mL) and equilibrate for a further 15 min. Discard the solutions after the 2nd equilibration.

Fig. 2

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 at least 4mL of cooling fluid onto the centre of the cooling plate.

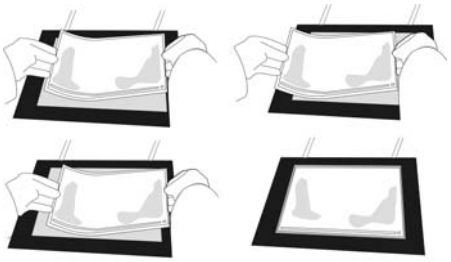

Fig. 3

7. Grip the gel (gel-side up) at the two lateral edges of the film, bend it into a "*U-shape*" and slide the film left and right to distribute the contact fluid evenly (Fig. 3). Remove any excess cooling fluid.
8. Place the the gel onto the cooling plate ensuring the IPG strip-slot is towards the cathode. The cathodal edge of the IPG strip-slot should match line "15" on the cooling plate.
9. Remove excess electrode buffer from wicks by tilting them vertically and dabbing on the paper pool bottom (fig. 4).

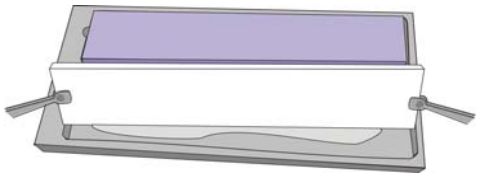

Fig. 4

10. Place the electrode stacks onto the gel edges overlapping the gels by at least 2 mm. Smooth out air bubbles with bent tip forceps.
11. Trim the film support of the IPG-strips on both sides to just beyond the gel edge.
12. Place the IPG-strip gel-side down, anodal side to the right, into the slot of the gel (fig. 5). 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.

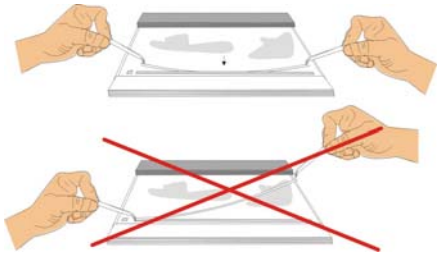

Fig. 5

13. Apply 5 µL SDS marker proteins to the well.
14. 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), start the run according to tables 2 or 3 (*2nd page*).
15. After 1 hour 10 min interrupt the run, remove the IPG strip(s) and continue the run.

Table 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

Table 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†	1500 V	50 mA ††	30 W	3 h 30 min

†valid for homogeneous gels, for the gradient gel 10-15 this phase 4 takes 4 h 30 min.
†† When gels are run in a FlatTop Tower, reduce the current to 40 mA / gel.

Table 3: Running condition over night for 1 homogeneous gel (15 °C)

Please note: It is **not** recommended to run the gradient gel (10 -15%) 2DGel flatbed over night.

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	220 V	5 mA	3 W	15 h