Equilibration and loading of IPG Strips on 2DGel flatbed

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. Prepare the two equilibration solutions from the **gel**company 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 completely.

2. Using a **gel**company equilibrator (fig. 1; cat. #1003-04) on an orbital shaker (30 rev/min) equilibrate each strip in DDT solution for 15 min. Then move the strip to a slot containing IAA solution (3 mL) and equilibrate for a further 15 min. After the 2nd equilibration discard the solutions.

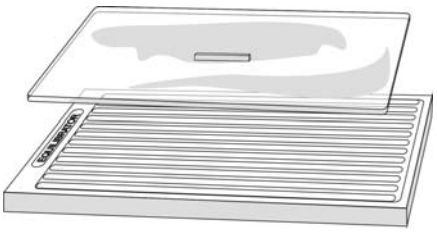


Fig. 1

3. Apply at least 2mL of cooling contact fluid (**gel**company; cat # PCC-150-TI) onto the cooling plate for good cooling contact.

4. Switch on the thermostatic circulator and set to 15 °C. To avoid condensation set the valve to "*by-pass*" so that the gel is not cooled at this stage.

5. 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. 2). Remove any excess cooling fluid.

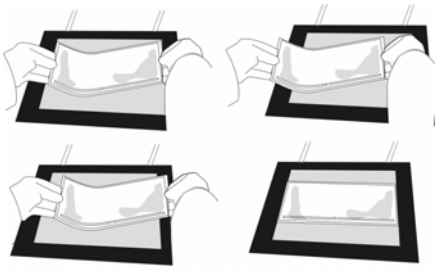
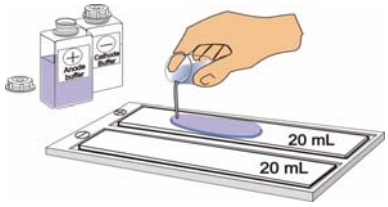


Fig. 2

7. 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 matching line "*15*" on the cooling plate.

8. Apply the anode buffer (20 mL) of to an electrode wick in a PaperPool (fig. 3; **gel**company; cat # 1003-03). Similarly, apply the cathode buffer (20 mL) to a separate wick.

Fig. 3



9. Place the electrode wicks onto the gel edges overlapping them by at least 2 mm. Hold wicks horizontally. Never at an angle as this would cause unequal buffer concentration along the wick. Smooth out air bubbles with bent tip forceps.

10. Trim the film support of the strips on both sides to just beyond the gel edge.

11. Place the IPG-strips gel-side facing down, anodal sides to the right, into the slots of the gel and push them carefully towards the anode edges of the slots (fig. 4). Slide along the backing of the strips with the forceps to ensure good contact to the bottom of the slots.

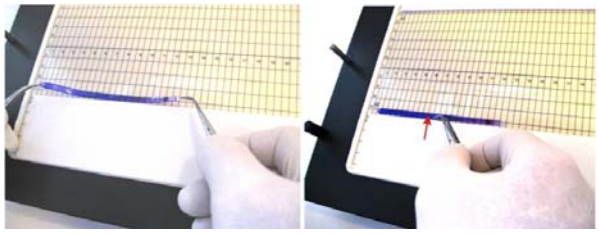


Fig. 4

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

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

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

Table 1: Preparing the equilibration buffers for 11 cm IPG strips:

Number of strips	Urea [g]	DTT [mg]	IAA [mg]	E. Buffer [mL]	Total volume [mL]
2	1.8	50	-	5	6
2	1.8	-	125	5	6
4	3.6	100	-	10	12
4	3.6	-	250	10	12
6	5.4	150	-	15	18
6	5.4	-	375	15	18
8	7.2	200	-	20	24
8	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	50 V	7 mA	1 W	30 min
phase 2	120 V	15 mA	3 W	30 min
phase 3	300 V	40 mA	10 W	10 min
after this step: remove the IPG strip!				
phase 4	1000 V	70 mA	40 W	1 h 30 min