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Instruction Manual

2D HPE™ Triple BlotGel NF 12.5 % Kit

Instruction Manual:
2D Electrophoresis and Blotting with 2D HPE™ Triple BlotGel Kit

General information

The gels can be easily blotted because the gels are non-covalently bound to the supporting film so that it can be removed from the gel after electrophoresis. This non-fluorescent (NF) supporting film also provides best results for fluorescent staining and labelling.

Suitable for running 3 x 7 cm IPG strips plus 2 marker lane by horizontal electrophoresis on HPE™ BlueTower, HPE™ BlueHorizon or Multiphor II™.

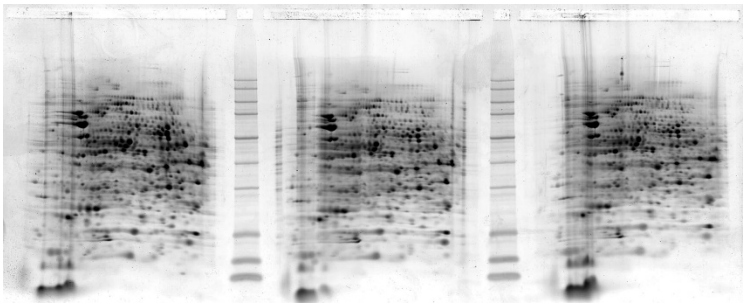


Fig. 1: Triple gel: 3x 7 cm IPG strips, Fluorescence stained

Kit components

4 plastic-backed gels, including running and equilibration buffers, FS wicks and cooling contact fluid.

Important before starting: Always wear powder free disposable gloves.

2D Electrophoresis – 2nd dimension SDS PAGE

Use only the SERVA buffer kit for the running buffers and equilibration solutions supplied with the gels!

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 Tab.1 and dissolve completely.

   - IAA solution: Weigh urea and iodoacetamide (IAA) and add the equilibration buffer according to Tab.1 and dissolve them completely.

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

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

2. Equilibrate each strip in (gel side up) in 3 ml (11 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

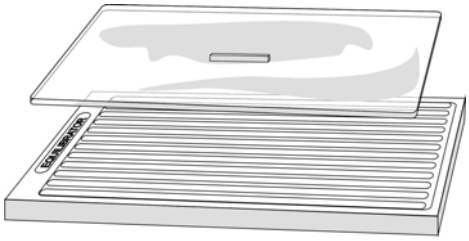


Fig. 2

3. After step 2 discard the solution.
4. Apply 40 ml of each electrode buffer to the respective electrode wick in the compartments of the PaperPool (Fig. 3).

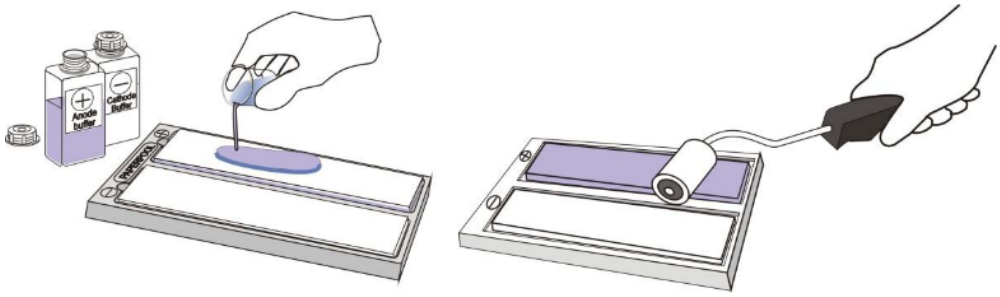
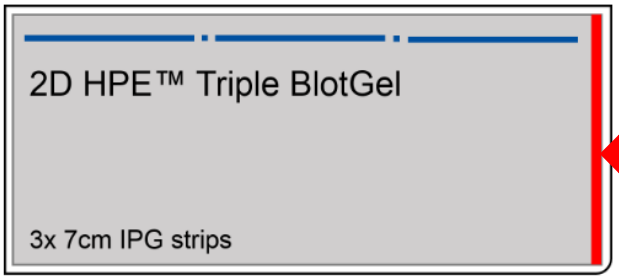


Fig. 3

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 “bypass“ to avoid water condensation on the gel surface.

7. Remove cover film from the side with the rounded edge (Fig. 4)



Adherent gel edge - Pull off the cover film from this side - After electrophoresis, separate the gel from this edge

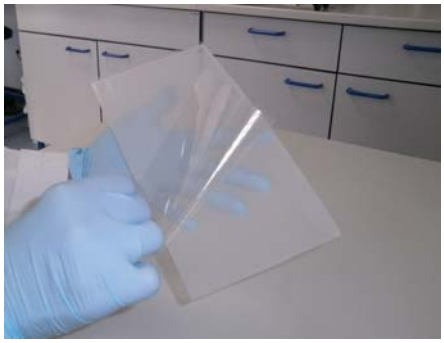


Fig. 4

8. 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. 5)

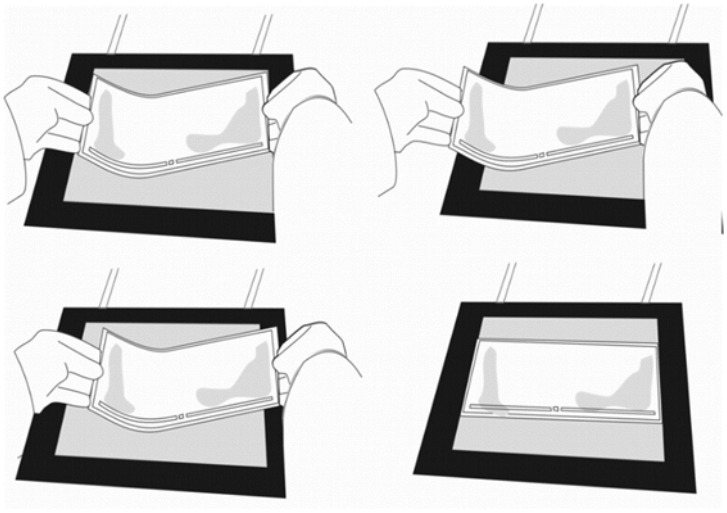


Fig. 5

9. Place the gel onto the cooling plate: the IPG strip-slot towards the cathode.
   NOTE: With the BlueHorizon, please use the gel positioning aid for the optimal positioning.

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).

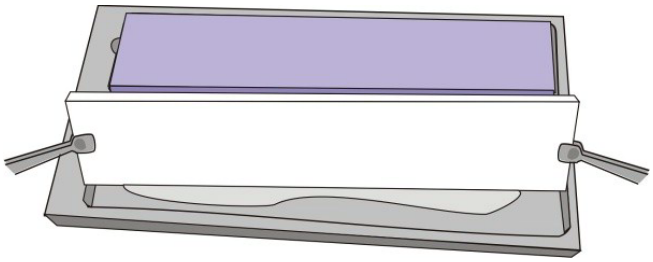


Fig. 6

11. Place the electrode wicks onto the gel edges overlapping them by at least 2 mm (Fig 7). Hold wicks horizontally! Never sloped, because this would cause unequal buffer concentration along the wick. Smooth out air bubbles with bent tip forceps.

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 SDS Gel and push them carefully towards the anode edges of the slots (Fig. 8). Slide along the backing of the strips with the forceps to ensure good contact to the bottom of the slots.

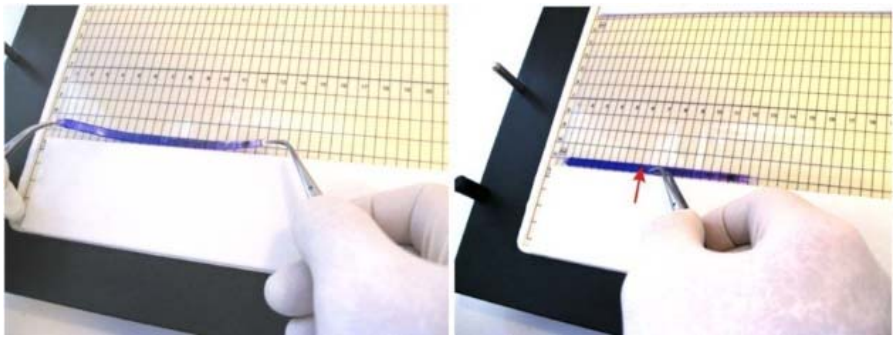


Fig. 8

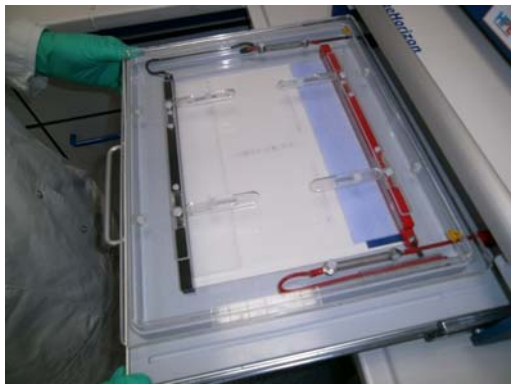
13. Apply 5 µl SDS marker proteins to the well.
14. Close the lid, lower the electrodes on the wicks the valve to cooling (15 °C), and start the run according to Tab. 2.
15. After 1 h 10 min interrupt the run (press “Pause“ or “Wait“ on the power supply), remove the IPG strips and then continue the run.

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

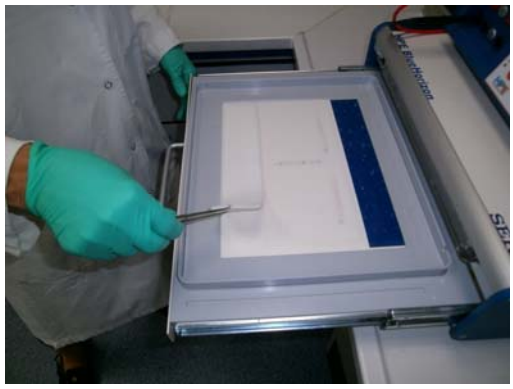
16. End of run:
    Open the drawer and the lid (Fig. 9a)

Fig. 9a



Carefully remove the electrode wicks (Fig. 9 b)

Fig. 9b



2D Electrophoresis – Blotting of the gel

1. **Semi Dry Transfer** – Preparation of the blotting fleece and the membrane

   - Wet each blotting fleece (2 per gel) and Connection Paper (1 per gel) with Transfer Buffer of choice.
   - Activate PVDF membrane.

2. **Semi Dry Transfer** – Preparation of the gel

   - Carefully remove HPE™ BlotGel with backing film from the cooling plate (Fig 10).

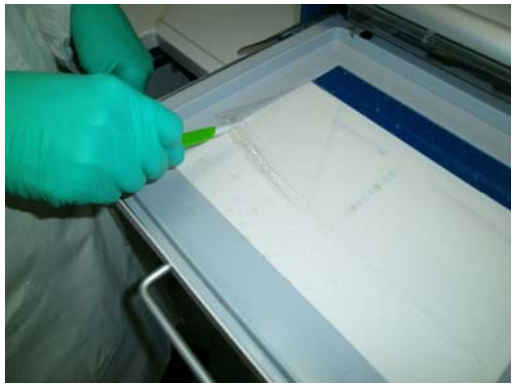


Fig. 10

   - Place HPE™ BlotGel on a glass plate (backing film down, Fig 11).

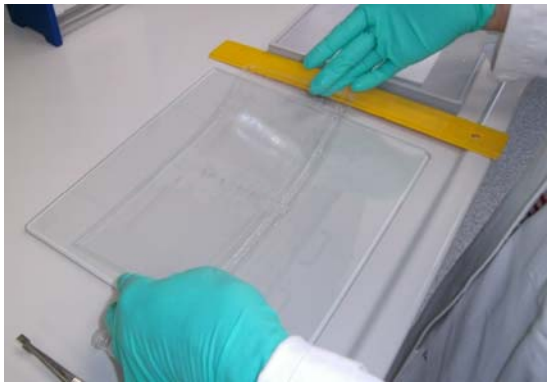


Fig. 11

- Remove adherent edge of the gel (Fig 12).

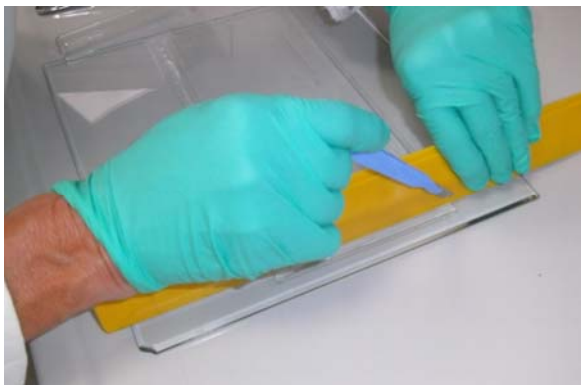


Fig. 12

3. **Semi Dry Transfer** – Building the blotting stack Part 1

- Place activated PVDF membrane on the gel (Fig 13).

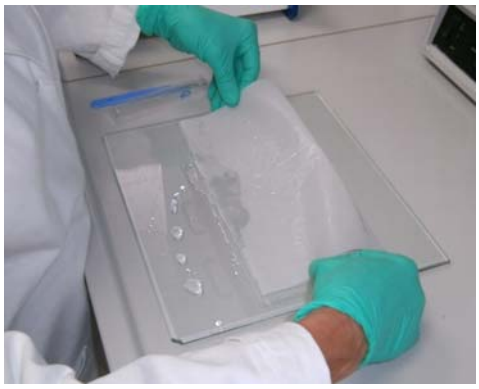


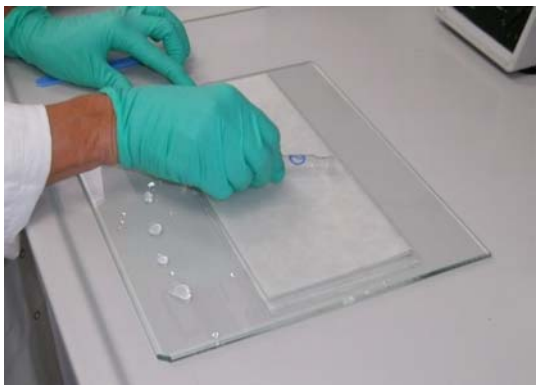
Fig. 13

- Add the pre-wetted Connection paper and blotting fleece on top of the stack (Fig 14).

Fig. 14

IMPORTANT: Avoid air-bubble within the stack.

Trapped air-bubbles can be removed by rolling a short glass pipet or test tube over stack.



4. **Semi Dry Transfer** – Transfer of the blotting stack onto the blotter

   - Lift the whole stack incl. gel and backing film from the glass plate (Fig 15).

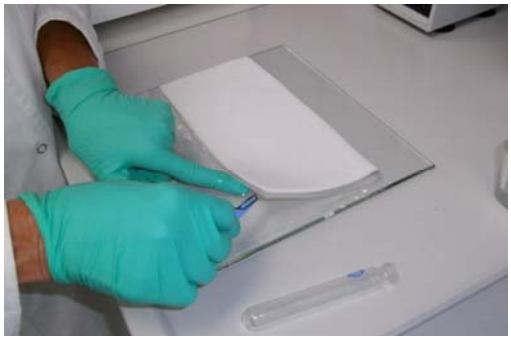


Fig. 15

   - Turn the stack – Backing film and gel are now on top of the sandwich (Fig 16).

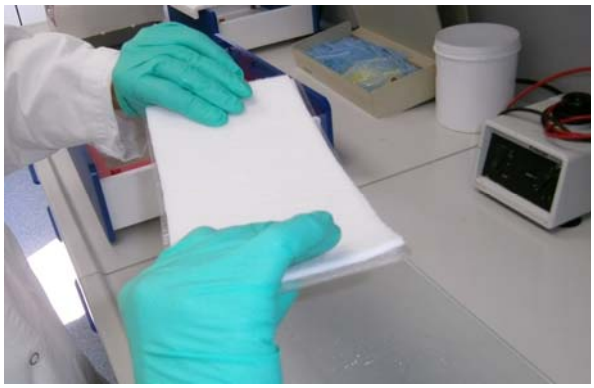


Fig. 16

- Place the stack on the blotter anode (Fig. 17)

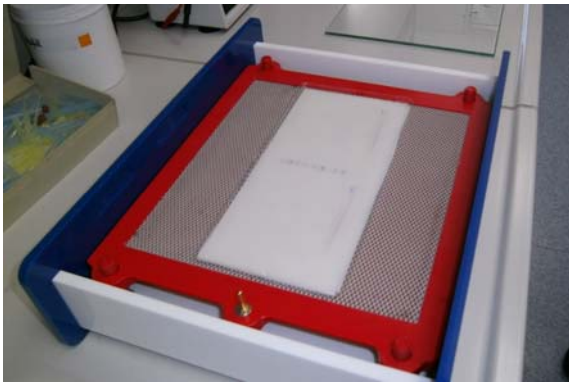


Fig. 17

5. **Semi Dry Transfer** – Removal of the backing film

- Carefully lift the edge of the film with a spatula (Fig 18).

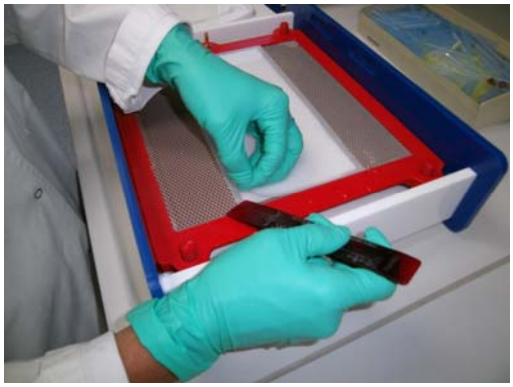


Fig. 18

- Pipet transfer buffer between film and gel (Fig. 19).

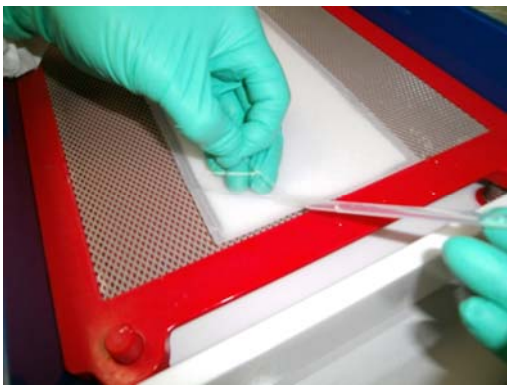
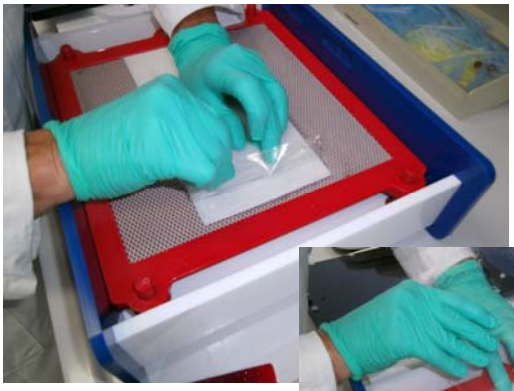


Fig. 19

- Carefully remove the backing film (Fig. 20).



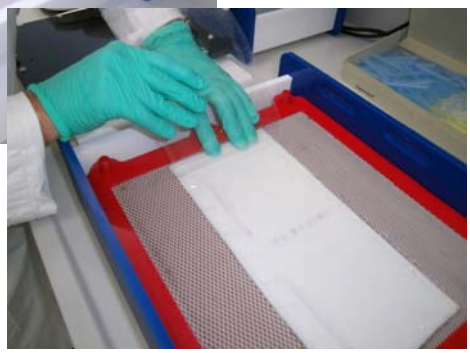


Fig. 20

6. Semi Dry Transfer – Building the blotting stack Part 2

- Place the pre-wetted blotting fleece air-bubble free on the HPE™ BlotGel (Fig. 21a - c).

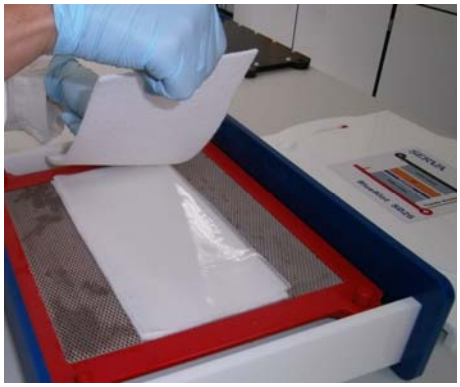


Fig. 21 a

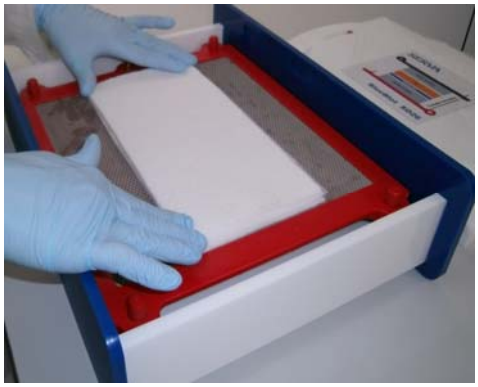


Fig. 21 b

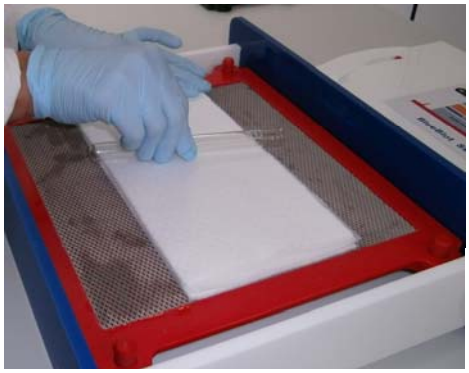


Fig. 21 c

IMPORTANT: Avoid air-bubble within the stack; Trapped air-bubbles can be removed by rolling a short glass pipet or test tube over stack (Fig 21 c).

- Carefully place the cathode on the blotting stack to avoid any shift of the stack (Fig. 22).

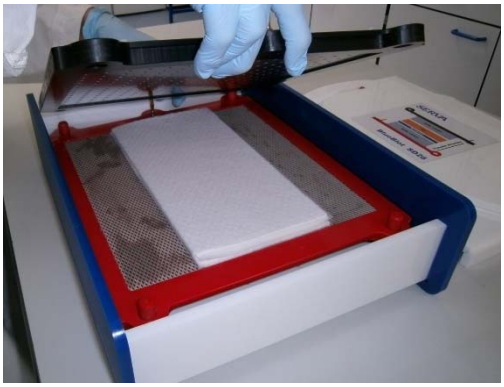


Fig. 22

- Close the lid and start transfer (Fig. 23). Choose the transfer settings according to the protocol given in the manual of the semi-dry blotter.

Fig. 23

7. NOTES

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