BlotGels-IEF

v7-07/20

Manual

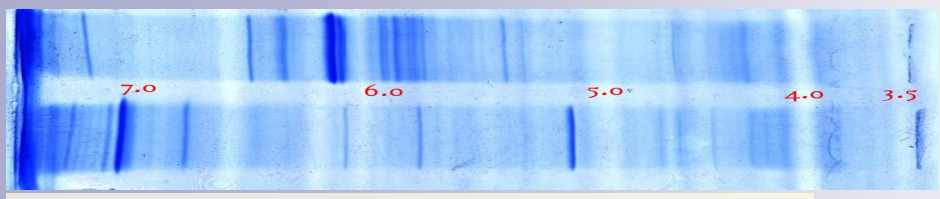


Fig.1a: BlotGel IEF 3-10 (IEF of pI Markers)

Fig.1b: (half) BlotGel on a blotting membrane, face down

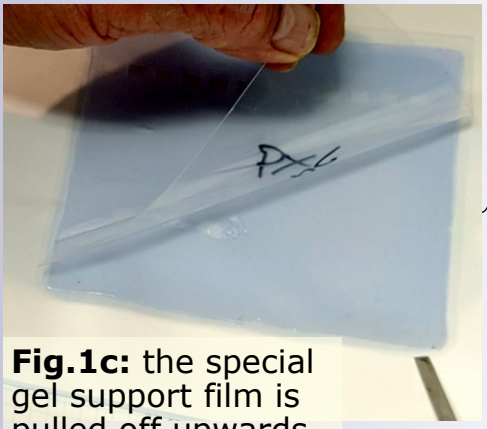


Fig.1c: the special gel support film is pulled off upwards

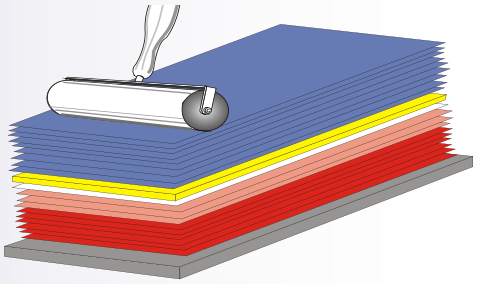


Fig.1e: semi-dry electro blot

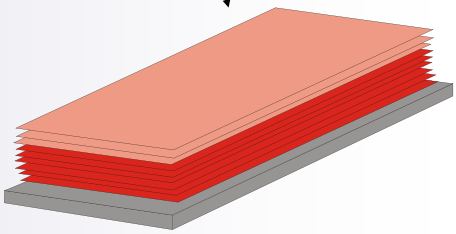


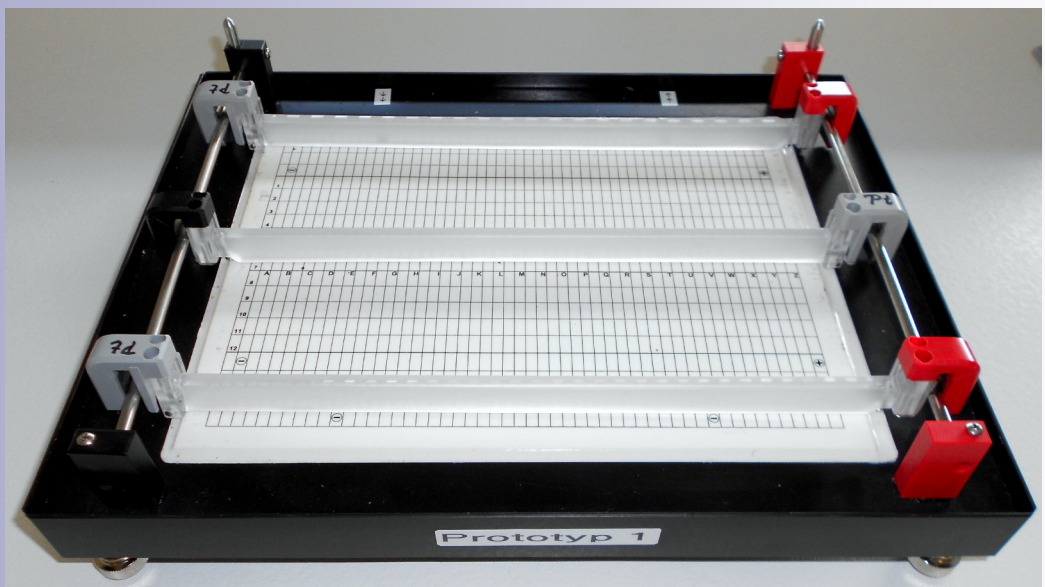
Fig.1d: semidry blot stack is waiting

General:
BlotGels have been polymerized to produce a matrix optimal for isoelectric focusing. IEFGels are thin gels (no slots: 0.5 mm/ with slots: 0.65 mm) with a gel concentration (T) of 4% and special cross-linking (C) of 3% delivering a special hydrophylic and soft matrix. Catalysts as well as other toxic and non-polymerized compounds are washed from the matrix resulting in gels that are non-toxic.
EDC's BlotGels are polymerized on a special support film: This film can be easily removed before the blot process: This enables electro blot procedures without any inconvenient film-cutting procedures.
Nylon nets are also not required! These would disrupt the electrophoretic separation processes and make them not recommendable in the case of the isoelectric focusing.
EDC's BlotGels contain a carrier ampholyte cocktail designed to achieve an optimal pH gradient. No electrode solutions and electrode strips are required, because the electrodes are placed directly onto the gel surface.
BlotGels are available with and without sample wells. For sample volumes see below.
For fluorescence visualizations all gels are on NF-films.

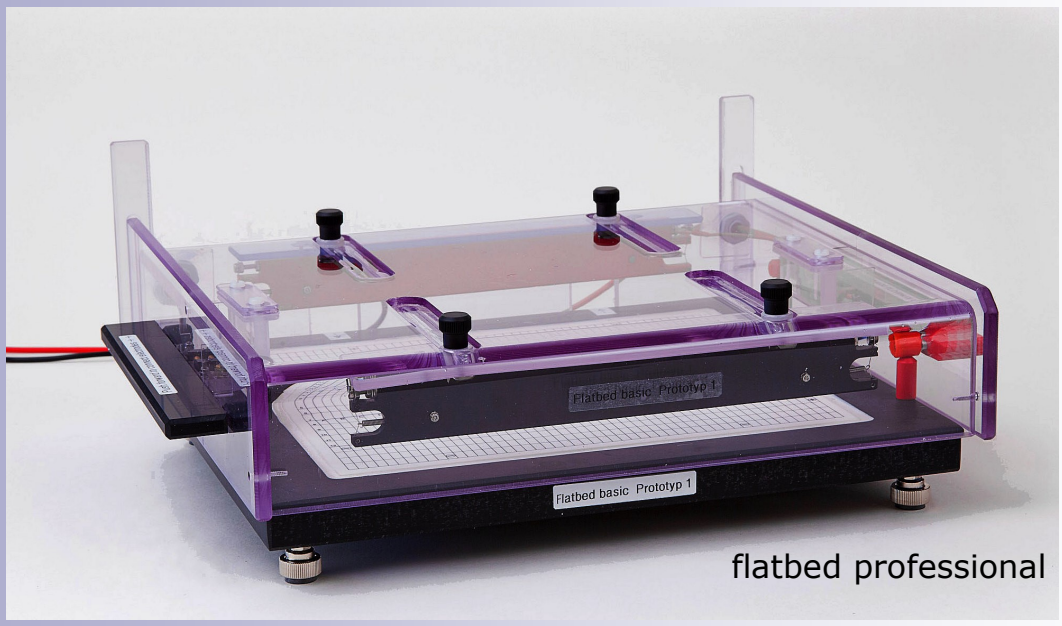
Gels (all gels are SoftGels NF *EQ-type*)
BlotGel IEF 3-10: 4 gels for native IEF, 0.5 mm, no slots *(edc-xxxx)*
BlotGel IEF 6-11 24S: 4 gels for native IEF, 0.65 mm, 24 slots à 20 µl *(edc-xxxx)*

Equipment

Power supply 2 kV, chambers: flatbed IEF professional (edc–IEF-2836), flat-bed basic (EDC: FB-2836), or Multiphor II (GE 18-1018-06).
Thermostatic Circulator: Huber minichiller 280
Immuno-Fluorescence: GE Healthcare Typhoon: (9200), Fuji: FLA 7000, 5100.
Rehydrating Tray for all immuno reactions (edc-3001)



flatbed professional with 3 electrodes (bidirectional IEF)



flatbed professional

Consumables and Chemicals

Cooling fluids: Cooling Medium (*edc-2011)* or Kerosene (*Serva 26940*)
Transfer buffers: Tris, epsilon-Aminocapron Acid, methanol, ethanol, acetic acid.

Sample Preparation

We recommend the use of IEF markers pH 4.6—10 from Merck.
Add an aliquot of the sample (25 µl) to each sample application piece/slot.

Loading the Gel

Unpacking the BlotGel

Open the bag with scissors and carefully remove the gel. Then – very carefully and slowly - take the protective cover-film from the gel surface.
Be aware that only a weak connection fixes the gel to its carrier film. Keep the cover film as it will serve as a protective sheet later. The gel is immediately ready for use.

Fig.2a: Pull off carefully the cover film

Gel-application: Spread 2.5 mL of kerosene onto the cooling plate of the focusing chamber to ensure good cooling contact, (Fig. 2b). Place the gel (gel side-up) on the center of the cooling plate (Fig. 3); avoid trapping any air bubbles. For the EDC flatbed basic and the flatbed IEF professional match the edges of the backing with the lines 4 and 16 (Fig. 3).

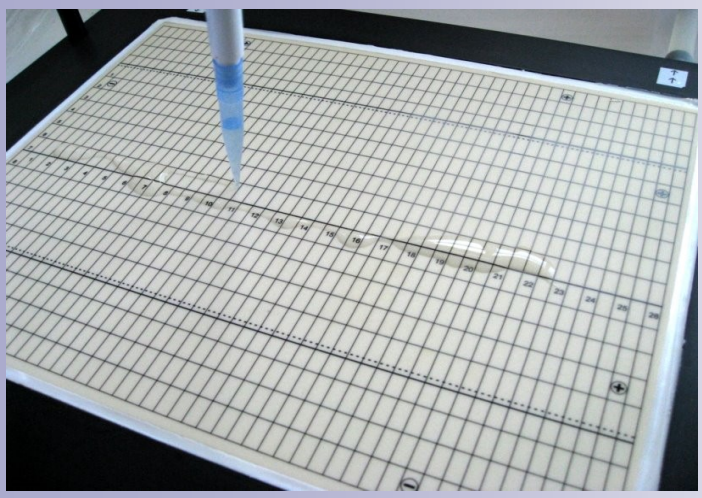


Fig. 2b: Applying kerosene onto the cooling plate

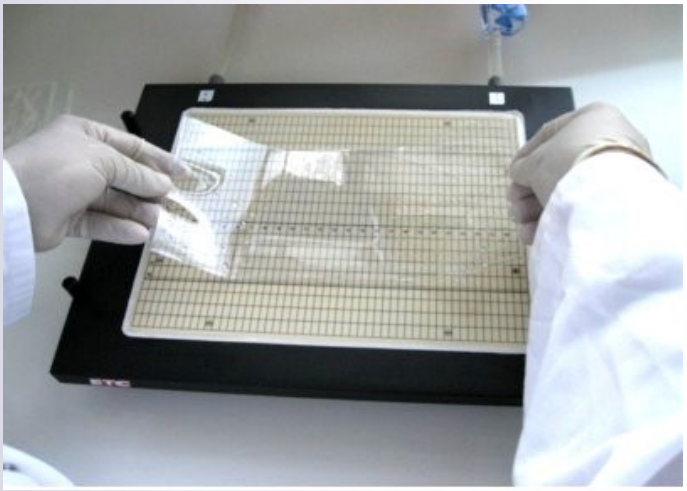


Fig. 3: Placing the gel onto the cooling plate

Sample application

Sample application on gels without slots can be performed using various types of commercially available sample applicators or silicone rubber masks. These allow different volumes to be applied.
For IEF it is important to apply the sample at the correct position within the pH gradient. For most samples the optimal application position is in the acidic region, close to the anode. For a new sample type the optimal position can be determined by a step trial test, (Fig. 4). It is important that the sample pieces have a distance of 1 cm from each other.

BlotGels IEF 24S, 40S, 104S: For some sample types, e.g. serum and CSF the position of the pre-formed wells is optimized for anodal application in a pH gradient e.g. 6-11 (Fig. 5). This well position might also be suitable for other sample types. The gels can also be turned around for cathodal application. Note: All wells must be filled with liquid; e.g. with sample or water.

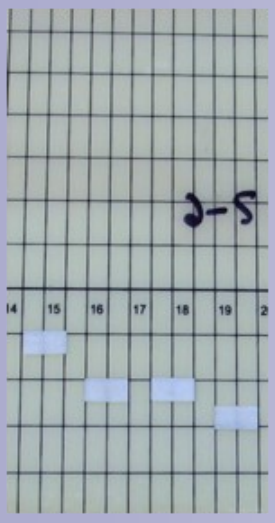


Fig. 4: Step trial test for optimization of sample application point.

Fig. 5: For serum and CSF the wells are orientated towards the anode

Isoelectric Focusing

Clean the platinum wires with moist tissue paper before (and after) IEF runs. Move the platinum electrodes to the correct positions over the edges of the gel. Lower the electrode holder onto the gel surface. The platinum wires should rest directly on the gel edges and not on the support film. Apply the samples, close the safety lid, and start focusing (Table 1 or Table 2). Note: There is no requirement to use electrode strips or buffers. Those must not be used.

Temperature: Isoelectric focusing has to be performed at a defined constant temperature because the pH gradient and the isoelectric points depend on the temperature. Switch on the thermostatic circulator, set to 7°C. Recommended temperature range: 5°C - 10°C.

Settings for Power Supply

During isoelectric focusing the electric resistance of the gel changes considerably and current or power values determine the voltage-values.Variations of the gel conductivity result in high variations of volt-values (Fig. 6.)

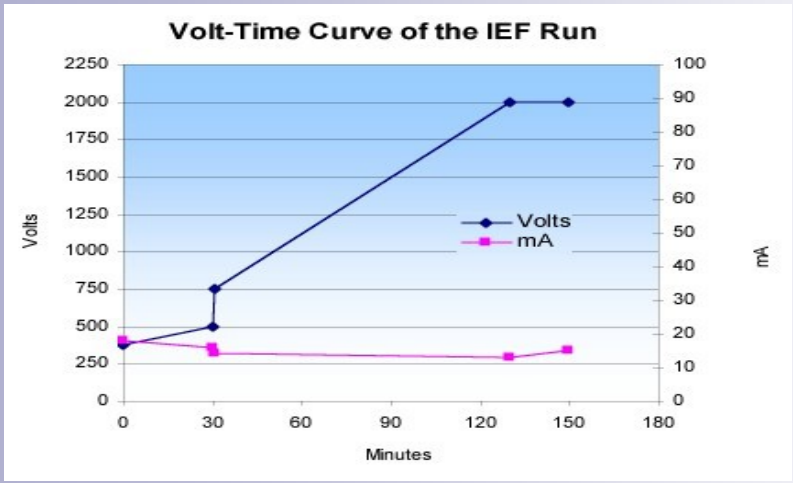


Fig. 6: Voltage / time curve for IEF

The most commonly applied method is to limit the voltage *via* the mA and the Watts achieved in the gel. With non-programmable (manual) power supplies use the settings in table 1. With programmable power supplies use the settings in Table 2.

Pre-Focusing: For some sample types it is beneficial to start with pre-focusing of the pH gradient before sample application. In this case add a step with the following settings to the beginning of the focusing:

1000 V 25 mA 10 W 20 min

Settings for Manual Power Supplies

With manual power supplies the electric conditions are limited by mA and Watts (Table. 1)

Table 1: Manual power supplies

Table 1	SET	*Start*[1]	SET*mA gel-thickness	SET*W gel-thickness	Time	Process
Step 1	500 V	*60 V*	0.5 mm: 6 mA 0.65 mm: 8 mA	0.5 mm: 5 W 0.65 mm: 10 W	30 min	sample entrance
Step 2	1650 V	*750 V*	0.5 mm: 12 mA 0.65 mm: 22 mA	0.5 mm: 10 W 0.65 mm: 15 W	90 min	focusing
Step 3	1800 V	*1850 V*	0.5 mm: 8 mA 0.65 mm: 22 mA	0.5 mm: 20 W 0.65 mm: 20 W	30 min	band sharpening

Note 1: At the start of each step the starting volt-value (in italics) should be adjusted *via* the limiting mA value.
Note 2: For a half gel apply the same voltage and half of the mA and W
Note 3: Remove sample applicators before staining.
*Note 4: Please select the values according to the gel thickness

Settings Programmable Power Supplies

With programmable power supplies the electric conditions are controlled by the programmed voltage curve (see Tab. 2). This is the better method but not all power supplies are programmable.

Table 2 Programmable power supplies

Table 2	SET	*Start Value*	SET	SET	Time	Process
Step 1	250 V —> 500 V	*(~20 mA)*	25 mA	15 W	30 min	sample entrance
Step 2	750 V —> 1650 V	*(25 mA)*	25 mA	20 W	90 min	focusing
Step 3	1800 V	*(~15 mA)*	15 mA	20 W	20 min	band sharpening

Note 1: Set "Ramping...on" or "Volt Level...Changing"
Note 2: mA and Watt should not be limiting
Note 3: Remove sample applicators before staining
Note 4: Half gels can run with this method without changing the settings

Removing the special support:

After the IEF procedure the gel support have to be removed in order to perform an electro blot. The use of a Nitrocellulose (NC) blot membrane is recommended.

Nitrocellulose (NC, reinforced): T

1. Cut the dry blot-film to the size of the gel (11 cm x 25 cm) plus 2 x 1 cm on the long side (=13 cm x 25 cm).
2. Fix the NC-film on the laboratory bench using a scotch tape. See figure 6.
3. Take the gel from the cooling plate and place it - face down - on the blotting membrane. Figure 7.
4. Press the gel bubblefree on the blotting membrane using a tissue and then a roller. Figure 8.
5. Wait for 3 minutes until the NC membrane becomes wet.
6. Grab one edge of the support and lift it slowly with a steep angle like in figures 9a and 9b. Use a spatula to hold down the gel itself if needed.
7. The electro-blot procedure can now be performed.

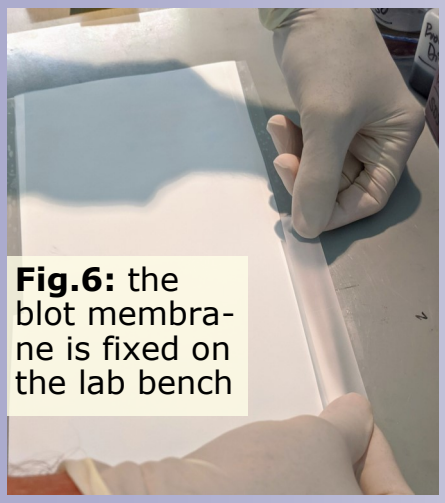


Fig.6: the blot membrane is fixed on the lab bench

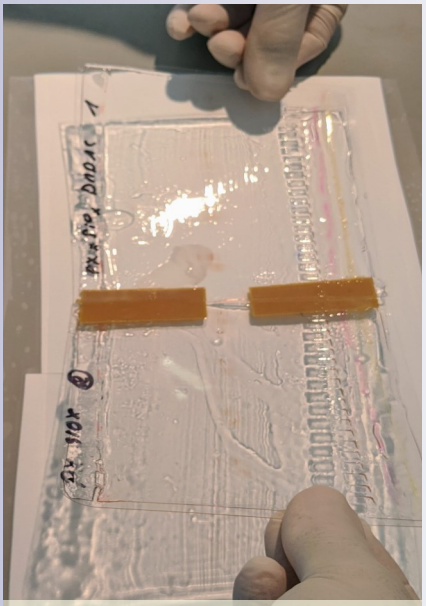


Fig.7: placing the gel on the blot membrane.

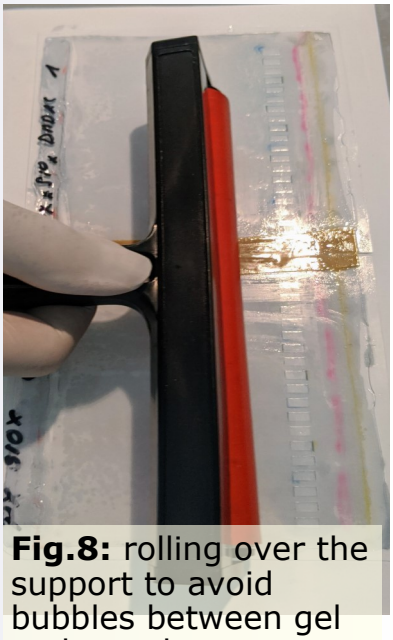


Fig.8: rolling over the support to avoid bubbles between gel and membrane.

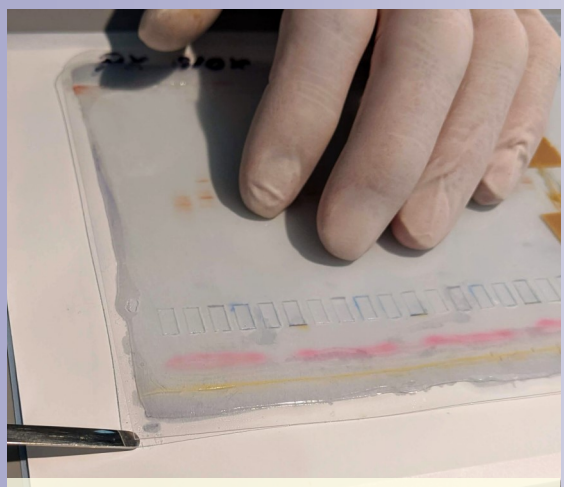


Fig.9a: BlotGel on a blotting membrane, face down. One edge is grapped with a spatula

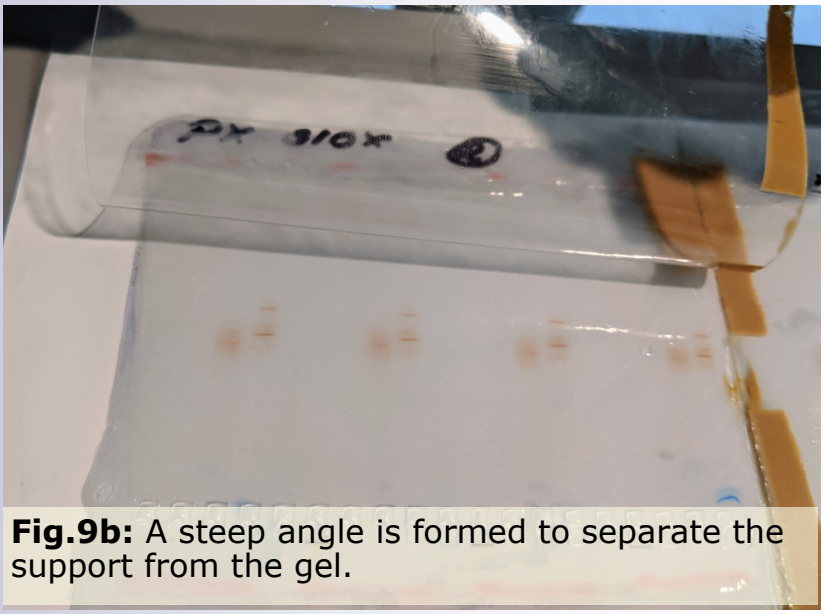


Fig.9b: A steep angle is formed to separate the support from the gel.

Semidry Blotting:

Cut the necessary filter papers (6 for the anode I, 3 for the anode II, 9 for the cathode) and the blotting membrane to the size of the gel (25 cm x 11.5 cm).
PVDF membranes must be activated for 10 minutes in 100% methanol. For Nitrocellulose-membranes this step is not necessary.

The blot-membrane and the gel must be equilibrated in blot buffer for 5 minutes, normally 400 ml Anode II buffer in the case of a following semidry blot procedure. See below.

- ⇒ Pour 200 ml of Anode II solution in the staining tray #1.
- ⇒ The BlotGel and the membrane must be equilibrated in 400 ml Anode II buffer for 5 minutes.
  The BlotGel together with its support film submerged with the support down.
  Place this staining tray on a rocking platform and shake slowly.
- ⇒ Now pour 200 ml of anode I solution into the staining tray #2;
- ⇒ slowly impregnate 6 filter papers in Anode I and place them on the anodal graphite plate.
- ⇒ When building a blot sandwich as described here, it is difficult to completely avoid air bubbles. They must therefore be pressed out with a roller: Start in the middle and roll out in all four directions.
  Press in such a way that the buffer in the sandwich oozes out but is not completely pressed out. When the roller is removed, the buffer should be "drawn" back in;
- ⇒ Slowly impregnate 3 filter papers in the Anode II solution and place them on the anode I batch;
- ⇒ Roll out air bubbles, see above
- ⇒ The transfer-unit can now be taken to the blot chamber. It is layed down on the paper -stack with the blotting membrane down. Avoid air bubbles and foldings.
- ⇒ Pour 200 ml of the cathode solution in the staining tray #3.
- ⇒ Soak 9 filter papers in the cathode buffer and place them on top of the stack
- ⇒ Roll out the air bubbles. Start in the middle and roll out in all four directions,
- ⇒ Wet the graphite cathode (black cable) with distilled water; remove the excess water with absorbent paper

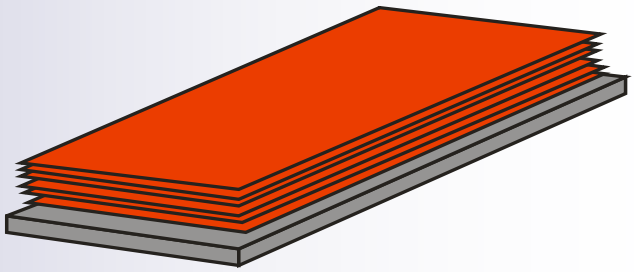


Fig.9: Anode I stack

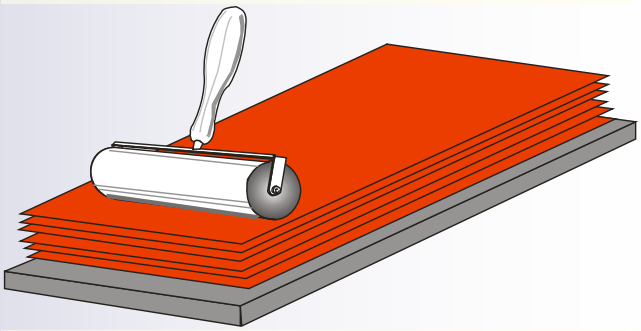


Fig.10: rolling over the Anode I stack

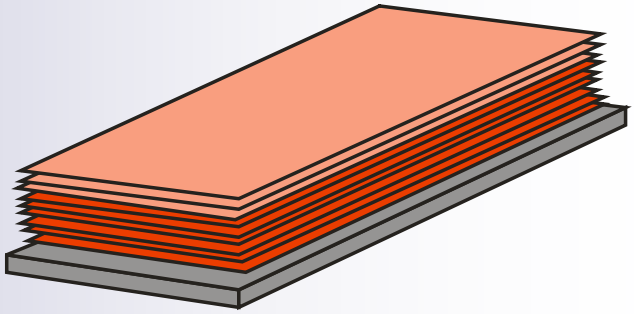


Fig.11: Anode II stack

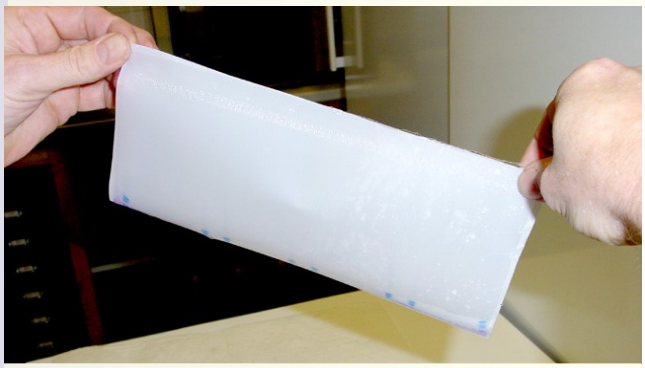


Fig.12: The membrane together with the gel is tranported to the blot stack.

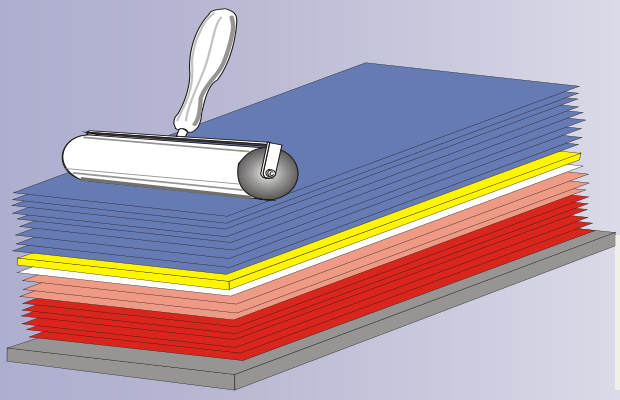


Fig.13: The complete blot stack: Anodal Graphite—Anode I—Anode II—Blot membrane—BlotGel—Cathode—Cathodal Graphite (missing)

Blotting Procedure:
Follow the instructions of your semidry blotter.

Conditions
Blot at a constant current:

0.8 mA/cm^2 =235 mA 1 hour or better 235 mAh for the whole BlotGel.

The blot does not warm up under these moderate conditions.
Higher currents result in a rise of the temperature of the gel and are not recommended.

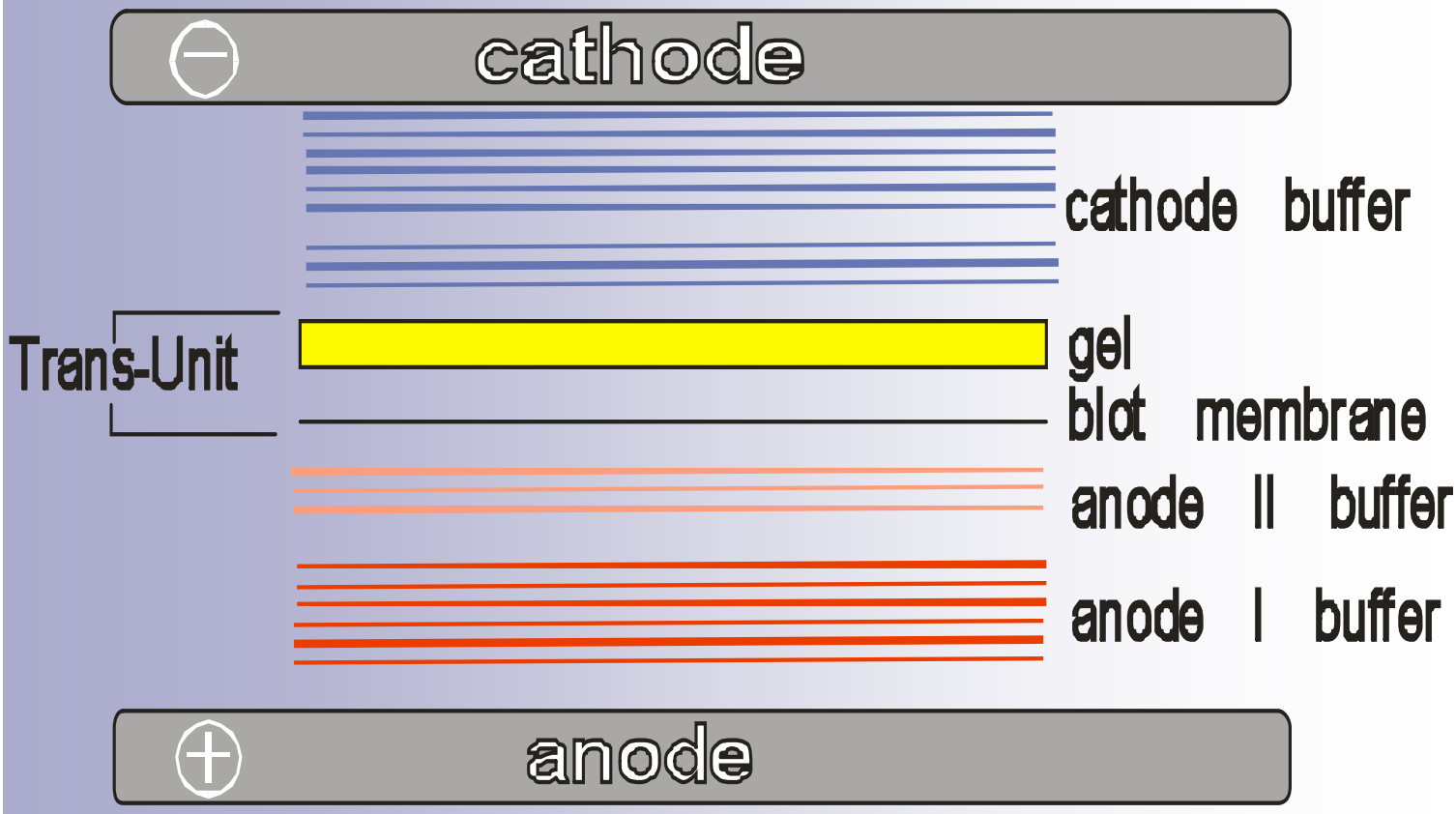


Fig.14: The complete semi dry blotting stack

Visualizations

Indian Ink staining: Unfortunately Indian Ink is no longer produced. However, the description of this fine method is no longer removed from this issue of the book for the case it would again be produced, or an ink with comparable performance would be found. The sensitivity comes close to the silver staining procedure.

PBS-Tween: 48.8 g of NaCl + 14.5 g of Na_2HPO_4 + 1.17 g of NaH_2PO_4 + 2.5 ml of Tween 20, fill up to 5 L with distilled water (pH 7.3).

⇒ Dunk PVDF-membranes in 100% methanol
⇒ Staining: 2 h or overnight with 250 ml of PBS Tween + 2.5 ml of acetic acid + 250 µl of fountain pen ink ("Pelikan Fount India"). Use a rocking platform, Filter the staining solution first.
⇒ Washing : 2 x2 min with water;
⇒ Drying: air-dry

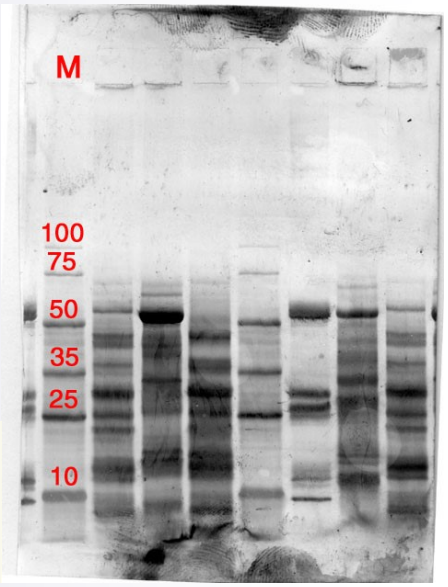


Fig.15: Indian Ink Staining overnight on Nitrocellulose

Ponceau Staining: This staining is less sensitive, but reversible: A so-called soft stain. Difficult to scan.

⇒ Dunk PVDF-membranes in 100% methanol for 3 minutes after the blot-procedure
⇒ Stain blot in 0.5% (w/v) Ponceau S red / 1% acetic acid/ 3% NaH_2PO_3 (add short before staining) / 0.05% Triton X 100, for 5 minutes.
⇒ Destain membrane submarine in hand-warm water for about 2 - 3 minutes. Caution: This can remove stain completely.
⇒ Drying: Hang the membrane in the air (see right picture) to dry for 25 minutes. This will develop fully the colour-contrast.

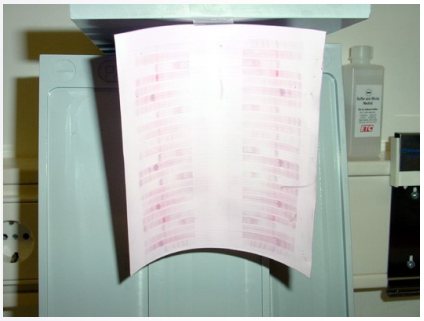


Fig.16: Hanging up the membrane for drying

Fast Green Staining:

⇒ Dunk PVDF-membranes in 100% methanol
⇒ Stain blot in 0.2 % (w/v) Fast Green / 1% acetic / 3% NaH_2PO_3 (add shortly before use)/ 0.05% Triton x 100 for 5 minutes.
⇒ First destain: move membrane submarine in water for about 5 minutes.
⇒ Second destain in methanol for about 2 minutes, bands will occur
⇒ Drying: Hang the membrane in the air to dry for 25 minutes. This will develop fully the colour-contrast.
⇒ Total (band) destain: Dilute 0.4 M NaOH 1 + 1 with methanol. Destain for 10 min..

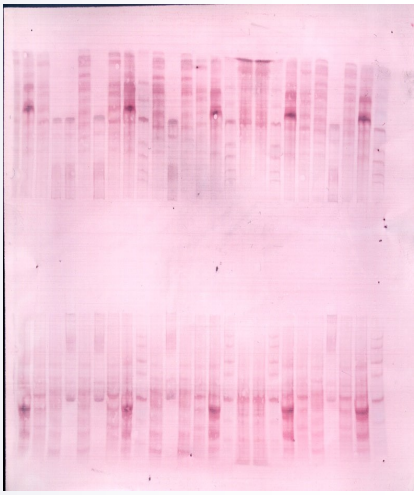


Fig.17: Ponceau S Staining PVDF

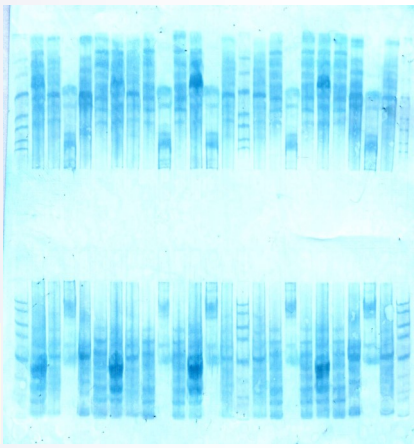
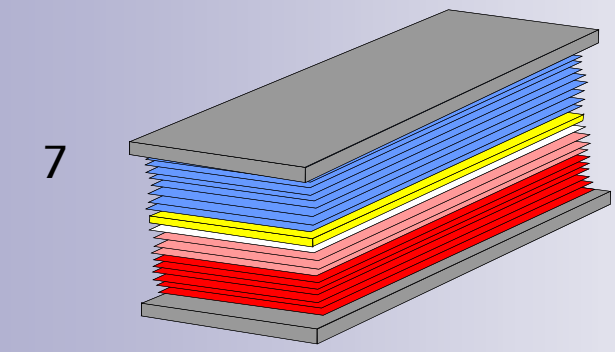
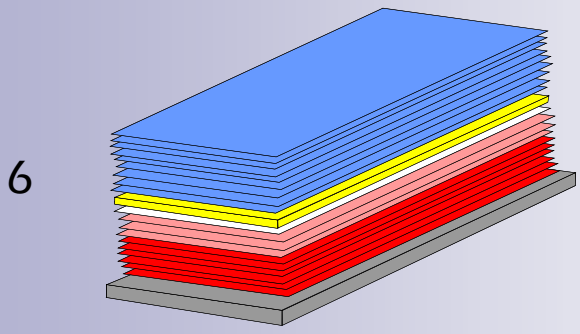


Fig.12: Fast Green Staining on PVDF

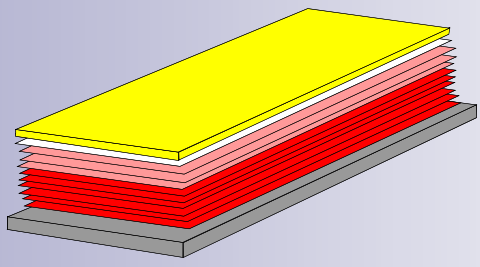
Stetup for Semi-dry Blotting



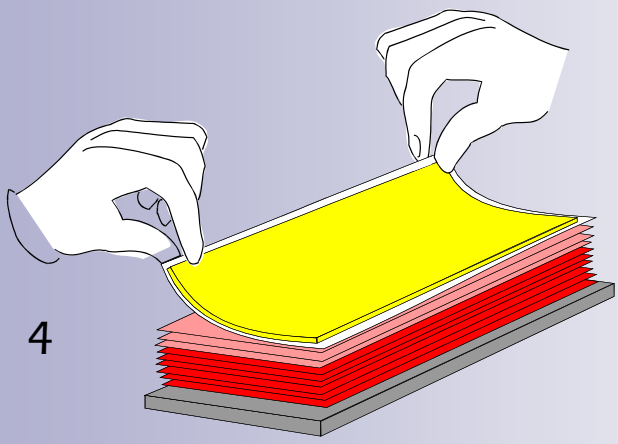
7 graphite cathode



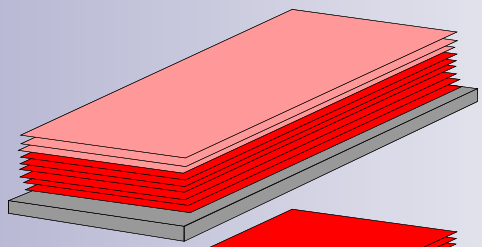
6 cathode buffer



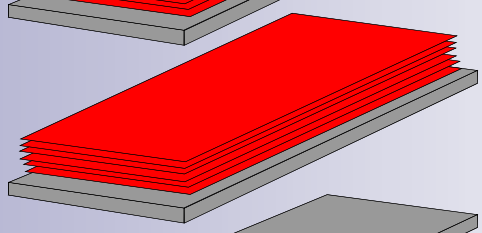
5 gel on the blot foil



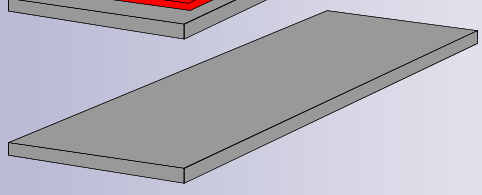
4 blot membrane together with the gel, blot membrane down



3 anode buffer II



2 anode buffer I



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