2D Kits Small *EQ-type*

V7 11/18

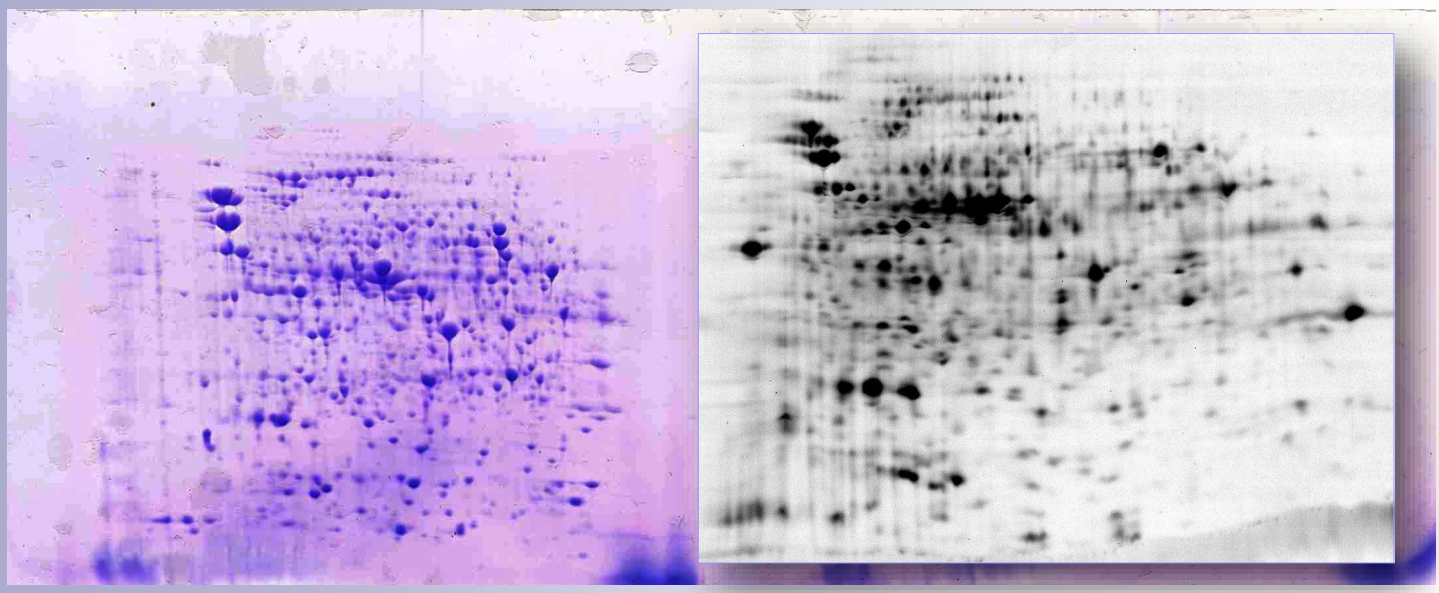
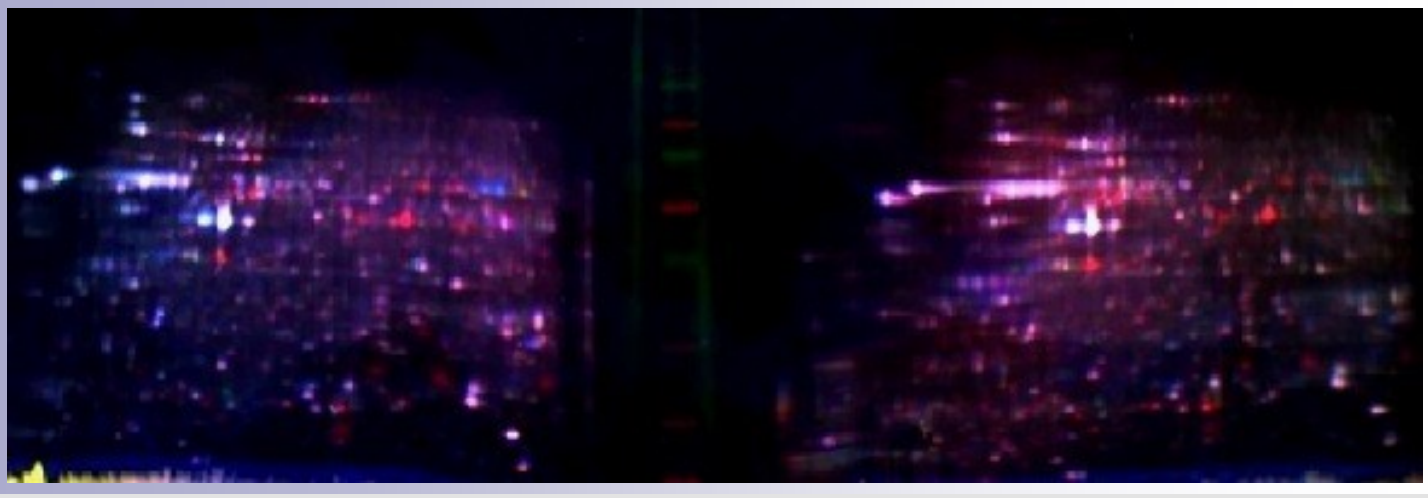


Fig.1: Left: *Coomassie-stained separations on PET-film support: 2 x 11 cm IPG-strips. Right: post-electrophoresis FITC-stained on NF-film. Below: Refraction-2D.*



***General**:*

ElphoGel 2D Kit is a Ready-To-Use gel kit and DryGel 2D Kit is a dry-gel kit for the second dimension (SDS) of 2D-electrophoresis on normal, heat resistent (Polyester), or non-fluorescent NF-support designed for horizontal flatbed systems.
Two 7 or 11 cm IPG-strips and one molecular weight marker are run on one gel.
The gel thickness is 0.5 mm, the gel size is 25 × 11 cm.
Polyacrylamide T-value is 12.5%. EQ-type is a special way of production.
Polyester-film:
The gels on polyester-film can be stained even with „Hot Coomassie Staining" technique of preparative runs and highly sensitive silver staining of analytical runs. Suitable for Pro-Q staining.
Because of some autofluorescence of the Polyester-film they are not suitable for fluorescence detection in blue and UV-channels.
NF-support:
Non-fluorescent support (NF). These gels should be used for fluorescence-techniques when all 3 channels are needed.
The fact that these gels can be scanned without any surrounding glass-cassette yields in higher sensitivity.
ElphoGels 2D:
The Ready-To-Use ElphoGels 2D can be stored at 4°/8°C for 6 month.

Equipment:
flatbed professional: horizontal chamber (edc-ief-2836) or flatbed modular: modular horizontal chamber (edc-fb-4856), flatbed large: horizontal chamber (edc-fb-3341).
Power Supply minimum 1000 V, 150 mA, 100 W; thermostatic circulator (lab supply)
Steel Staining Tray: (stainless steel tray with elevated stainless steel grid) (edc-wm-n1)
optional: Multi 6 Tray: for staining 6 gels simultanous (edc-wm-m6)

Accessories:
DryPool Combi (*edc-me-d*)
Tray for rehydration of dry gels (only for DryGels) and soaking electrode strips

2D-Gel Kits:
ElphoGel 2D Small *EQ-type*, 4 gels, for 2 × 7 or 11 cm IPG each (edc-3052)
ElphoGel 2D Small NF *EQ-type*:, 4 gels, for 2 × 7 or 11 cm IPG each (edc-3152)
including:
4 gels, 4 x 2 electrode-gels, 50 ml equilibr. buffer (IPG-strips!), 25 ml Cooling Fluid, 4 preserving sheets. Only for the DryGel Kits: gel buffer (250 ml), 4 drying cardboards.

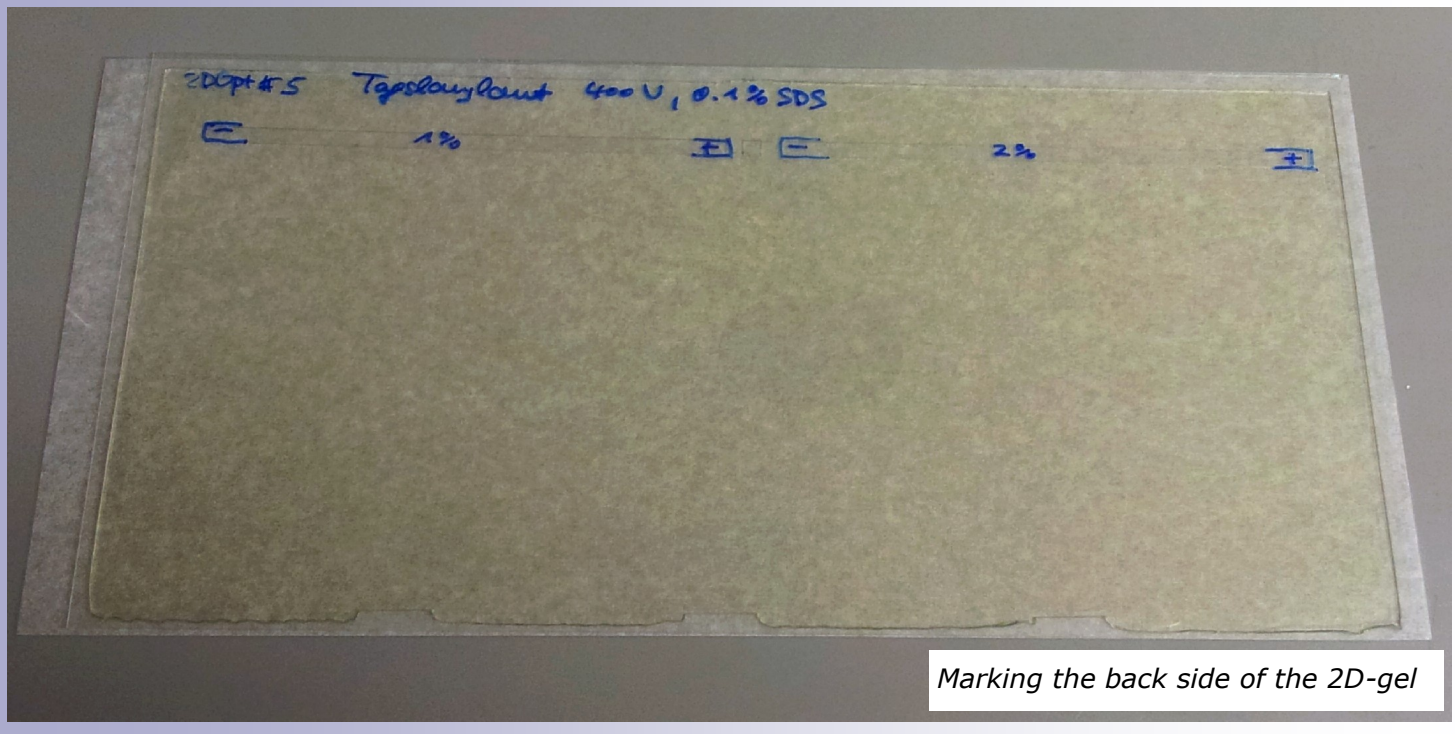
Additionally Required: SDS-standard, Urea, Dithiothreitol (DTT), Iodoacetamide (IAA)

Marking the 2D-gel's backside (with a waterproof pencil)
Film-supported gels have the advantage of writing something on the support's back side. This helps the experimenter orientating while processing the IPG-strips.

1. The 2 IPG-numbers or the experiment name.
2. The orientation of the IPG-strips (the pH-values)
3. The ends of the IPG-slots (for targeting the IPG-strips into the slots)

This ensures not to mix up the 2 strips among themselves and their orientation on the gel. The frames at the end of the slots help to hit the strips to the ends of their slots.

This can be done whith the dry gel or with the rehydrated gel. Lay the rehydrated gel onto the (clean) preserving sheets, gel side down.



Marking the back side of the 2D-gel

Application of the gel onto the cooling plate *(DryGels and ElphoGels)*
Take a pair of scissors and cut the plastic bag of the dry gel open. Take out the gel and locate the side of the dry gel. Figure 4.
To avoid water condensation on the gel surface: Do not switch on the thermostatic circulator at this stage or set the valve at the tubing to „by-pass".
Apply a very thin layer of the cooling fluid (2 ml) onto the cooling plate with a pipette (Fig.5), in order to ensure good cooling contact.
Place the gel (film down) onto the center of the cooling plate: The side with the slots is orientated towards the cathode (-). First the middle of the support-film should be contacting the cooling fluid.
Wipe the film over the cooling fluid to distribute the cooling fluid evenly on the area later covered by the gel.
Figure 6.
Then lower the two ends of the film onto the cooling fluid, avoid trapping air-bubbles.

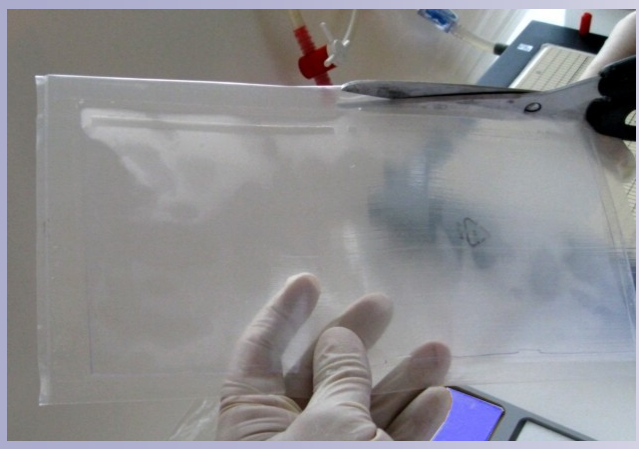


Fig.4: Cutting the plastic bag

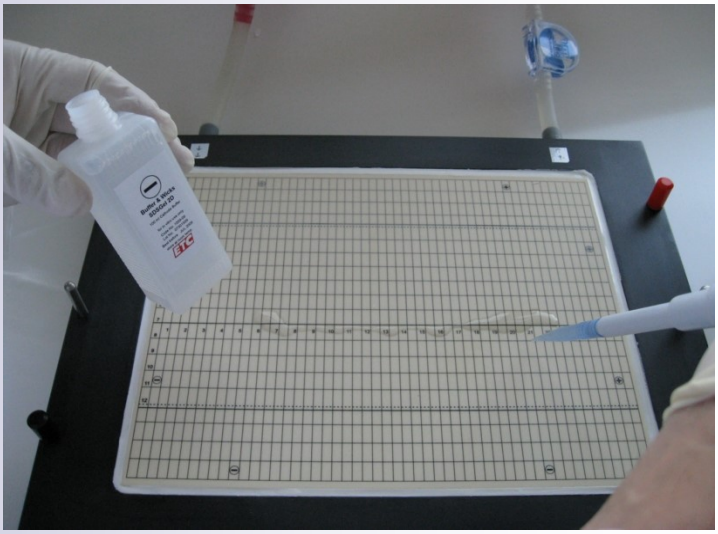


Fig.5: *Apply 2.5 ml cooling fluid as cool contact fluid onto the cooling plate*

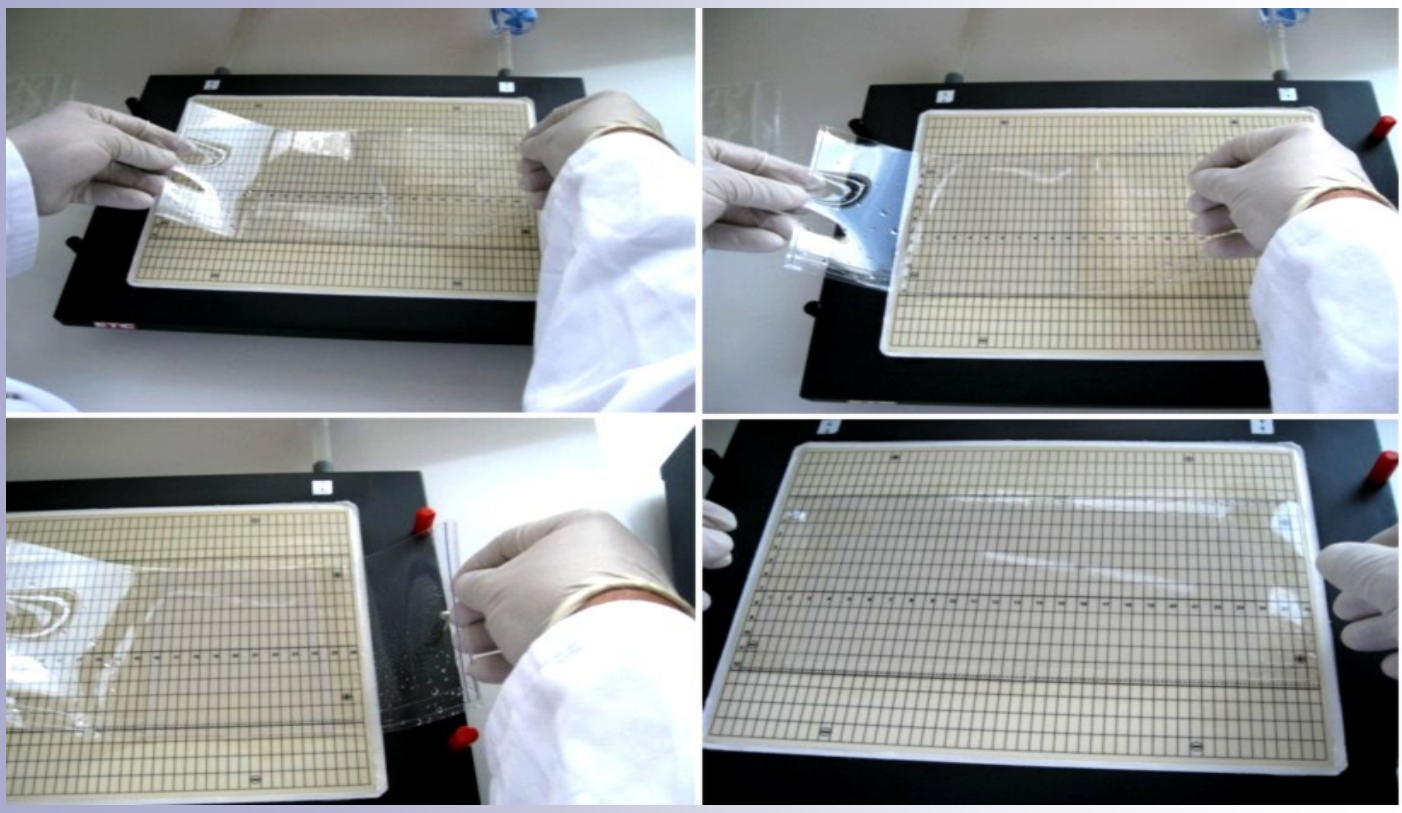


Fig.6: *Slide the film-support left and right on the cooling plate to distribute the cool contact fluid evenly.*

Starting the Electrophoresis

SDS - Marker: Dilute the SDS-standard at least 1 + 1 with the gel buffer.
IPG-Strip Equilibration: in 5 ml equilibration buffer per 7/11 cm long strip.
Important: Use EDC's IPG-strip equil. buffer, res. gel-rehydration buffer.
1, 2: Weigh in the reagents, add equilibration buffer, and dissolve them completely

Step	Urea	DTT	IAA	equilibr. Buffer	Time
1	1.5 g*	40 mg*	- - -	add 4.2 ml**	15 min
2	1.5 g*	- - -	105 mg*	add 4.2 ml**	15 min

* per strip
** makes 5 ml

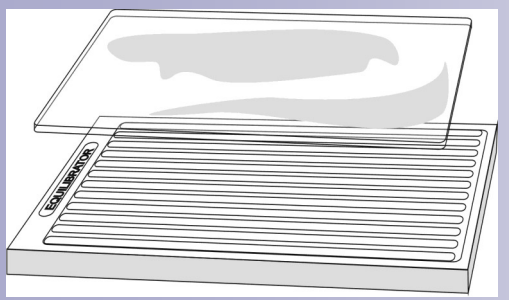


Fig. 9a: Equilibration steps 1 and 2

Fig. 9b: Step 3, shaking strip with gel buffer

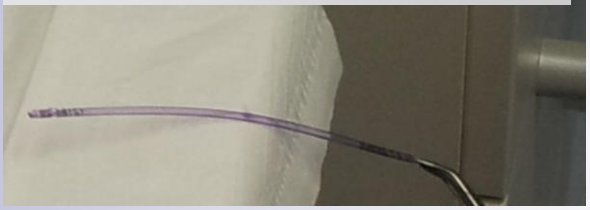


Fig. 9c: Wipe away exceed buffer volume from the backside

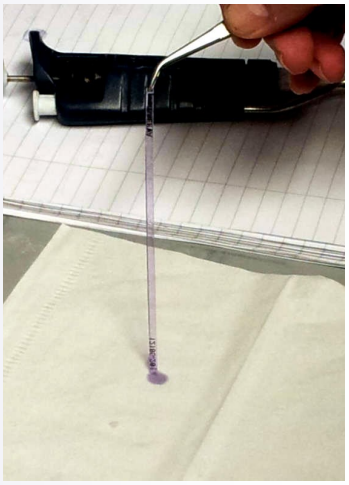


Fig. 9d: The last droplet is soaked by a paper tissue

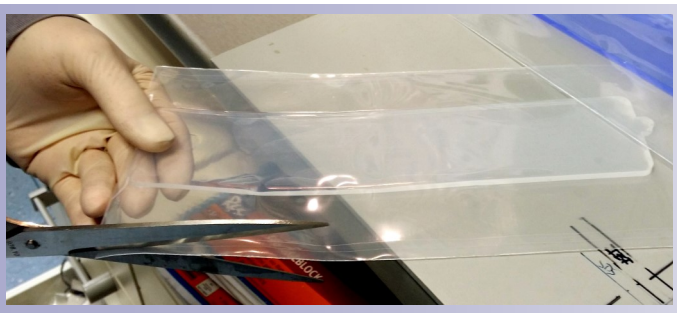


Fig. 5: *Cutting out the electrode gels out of their bags.*

Use gloves and clean forceps!
Cut out the electrodes out of their bags, fig 5.
Carefully grap the electrode gels and put them onto the gels with an overlapping zone of ~3-4 mm. Fig. 5, 6, 7 and 8.

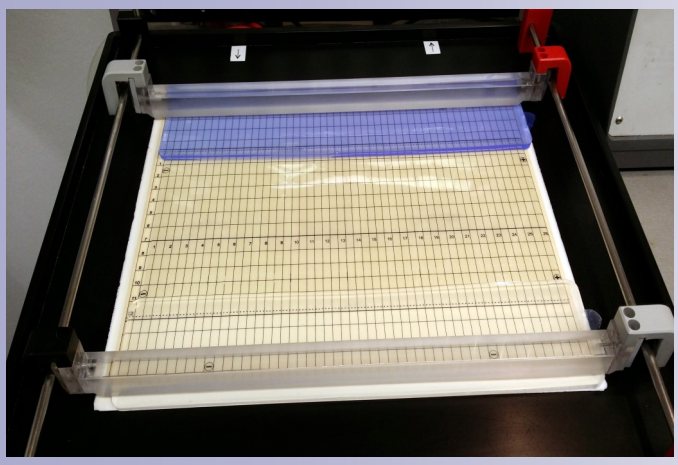


Fig.8: *2D-Small with its gel-electrodes on flatbed professional's cooling plate*

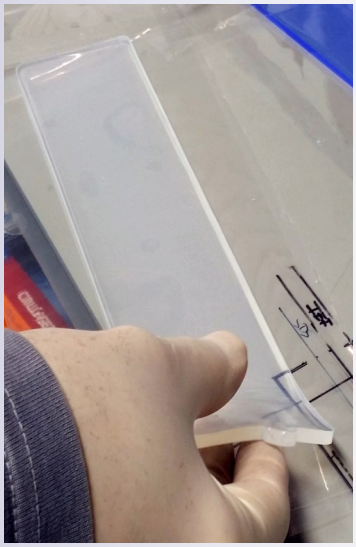


Fig.6 *Grap the electrode….*

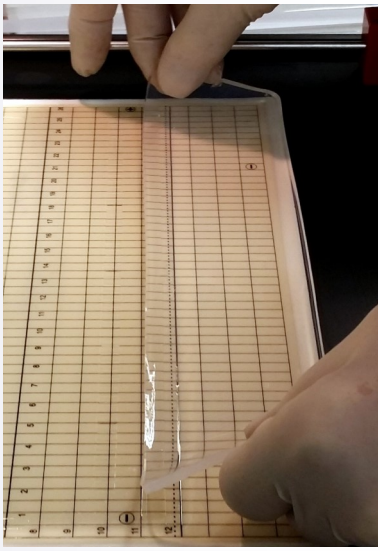


Fig.7: *...and place it onto the gel's edge*

Application of the IPG-strips

Wipe exceed buffer from the IPG's backside, fig. 9c + d. The film support of the strips should be trimmed on both sides to protrude only by 2 mm. Then the IPG-strip film will not cover the slot for the SDS-standard.
Using two forceps place each strip gel-side down (!) into the slots of the SDS-gel and push them carefully towards the anode to ensure good contact to the anodal edge of the slot, Figure 10a. Slide along the backside of the strips with the forceps to ensure good contact to the gel. Anodal end (+) of each strip should be orientated to the right hand side (international agreement).

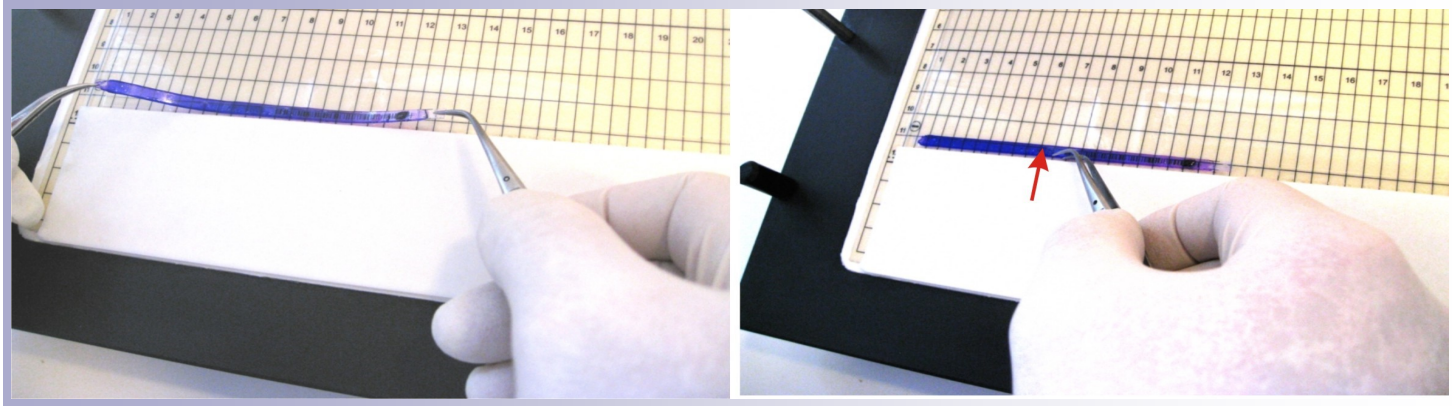


Fig. 10a: *Placing the IPG strip into the slot, and pushing it carefully towards the anodal edge of the slot.*

Pipetting the marker solution

Pipet 5 µl of the SDS-standard into the standard well. Figure 10.

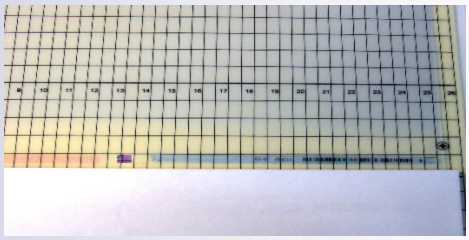


Fig. 10b: *Arrangememt of the 2DGel Flat-bed , cathodal buffer strip, the IPG-strips and the marker slot.*

Setting up the Electrode Lid and starting of the electrophoresis

flatbed basic and large: Place the lid on the elevated park position. Adjust the electrodes to the outer edges of the electrode wicks, Figure 11.
Push the 2 handles forward and lower the lid until the platinum electrodes sit on the electrode papers.
Switch on the thermostatic circulator or set the valve to „pump through" and start the power supply with the conditions given below.
flatbed modular: First pull out the draw totally, then do the described handlings.

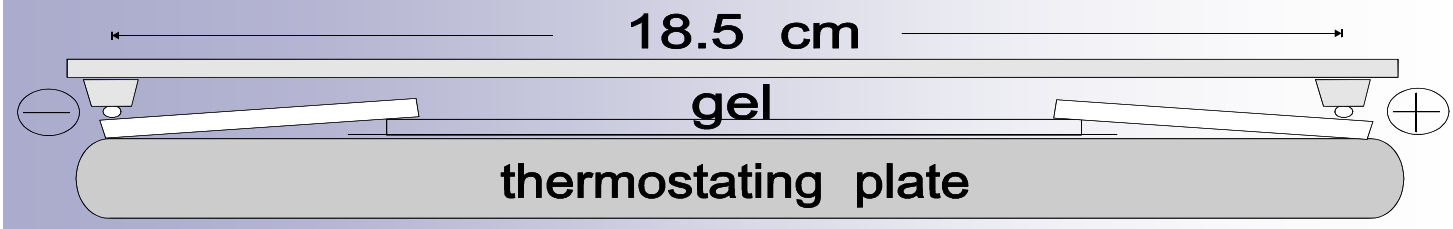


Fig.11: *Horizontal electrophoresis chamber. Arrangement of gel, buffer wicks and electrodes*

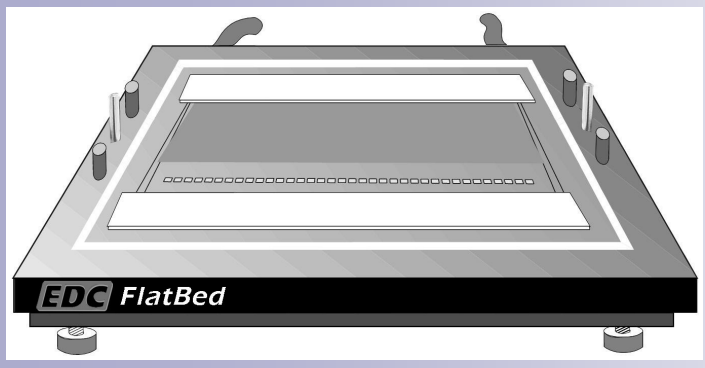


Fig.12a & 12b: *Horizontal electrophoresis chambers: Arrangement of gel, buffer-wicks and electrodes*

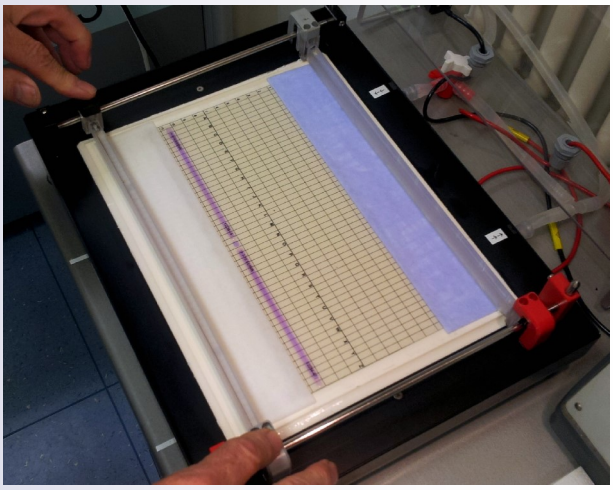
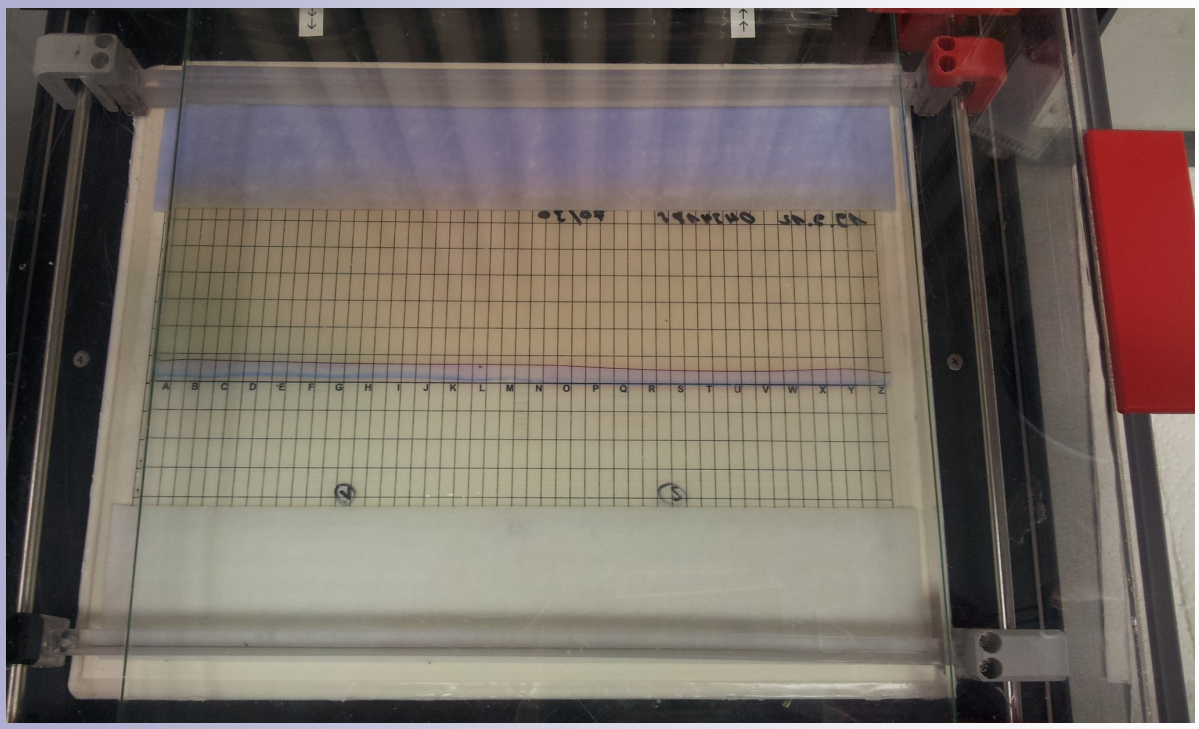


Fig.12c: *….the run*



Running conditions (15°C)

There are three different ways to run a this 2D-gel:

1. ***Day Run*** (5 hours)
2. ***Overnight Run*** (19.5 hours)

Power supply settings per gel

The three phases apply low electric fields to transfer the proteins from the IPG-strips into the SDS-gel. When there is written: **remove the strips!**: press „Pause", open the lid and remove the IPG strips. The IPG-strips will destroy the gel when staying in place during the whole run

Then close the lid and continue the separation.

flatbed modular: Push the draw in to totally to get contact to the power supply.

1. Day Run

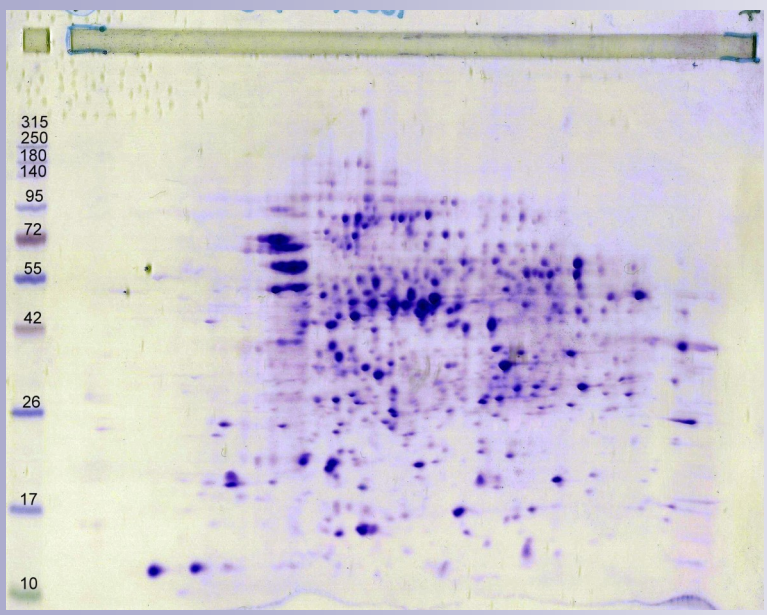
Ready within ~3 hours.

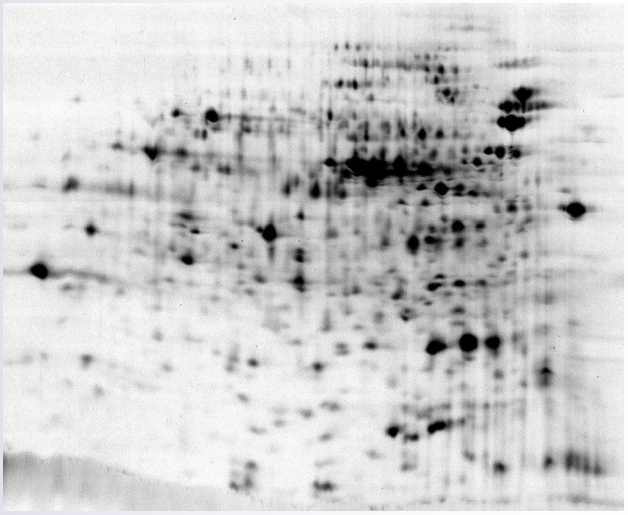
Total running time: 3 hours

Bio-Rad Power Pac HV: type in this parameter as constant —-> n mA
(Select „BASIC" mode) type in the other settings as limits —- n V

1 Gel:	Set V	*Start Value*	SET mA	Set W	Time	Comment
phase 1 (sample entrance 1)	100 V	*(~40 V)*	7 mA	2 W	30 min	Discontinuity ~5 mm before the slot
phase 2 (sample entrance 2)	150 V	*(~90 V)*	13 mA	3 W	30 min	Discontinuity through the slot
phase 3 (sample concentration)	200 V	*(~160 V)*	20 mA	5 W	10 min	after this phase: **remove the strips!**
phase 4 (main electrophoresis)	650 V	*(~310 V)*	35 mA	25 W	110 min	Bromophenolred + SDS -zone in the Anode

Optimal shut-down of the run: See page 10





Day Run: nearly no smiling. spot shape Okay.

2. Overnight Run

Runs overnight (~18 hours). Smiling is cero. Spot-shape Okay. Uses night time.
Do not lower the temperature to 10°C (water condensation)!

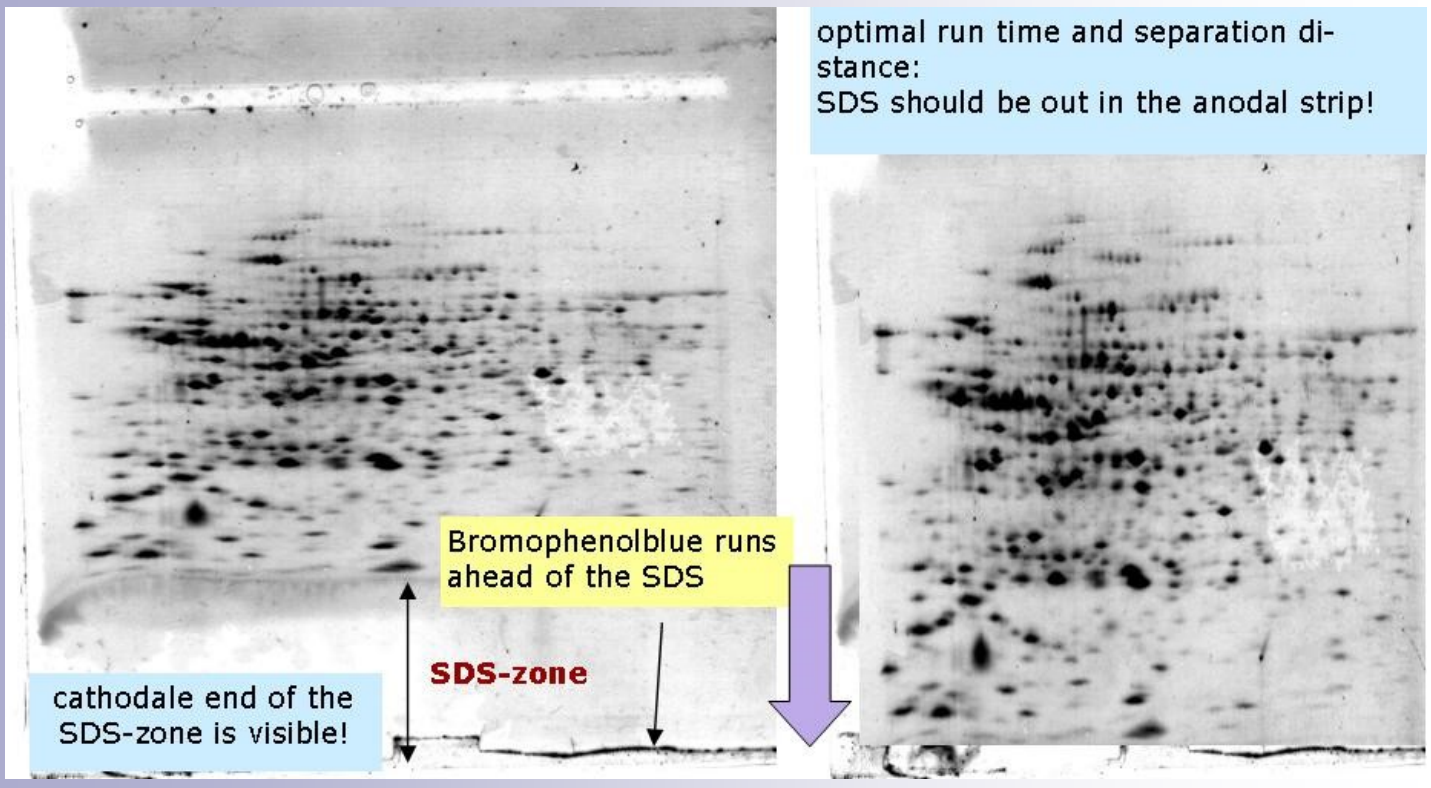
Total running time:~18 hours

1 Gel:	Set V	*Start Value*	SET mA	Set W	Time	Comment
phase 1 (sample entrance 1)	100 V	*(~40 V)*	7 mA	2 W	30 min	Discontinuity ~5 mm before the slot
phase 2 (sample entrance 2)	150 V	*(~90 V)*	13 mA	3 W	30 min	Discontinuity through the slot
phase 3 (sample concentration)	250 V	*(~160 V)*	20 mA	5 W	10 min	after this phase: **remove the strips!**
phase 4 (main electrophoresis)	100 V	*(~100 V)*	6 mA	2 W	1020 min *(17 h)*	Bromophenolred + SDS -zone in the Anode

Optimal shut-down of the run: See next page.

Optimal shutdown

The (andodal ion) SDS should be completely in the anodal strip!



The (andodal ion) SDS should be completely in the anodal strip!
In EDC's SDS buffer-system the SDS runs behind the Bromophenolblue.
This means: Stopping the run with the BPB will left ~1.5 cm SDS in the separation gel.
Remedy:
Let the SDS run out of the gel and use the separation distance for your proteins by optimized shutdown:

a) You can see the backside of the SDS-zone, marked red by the Conchenille dye from EDC's Sample Buffer. Let this zone out in the anodal strip, then shutdown.
b) Use a prestained standard and run it till the 10 kDa stays just before the anodal strip.

Semi-Colloidal Coomassie Staining Protocol

Download this protocol:
http://www.electrophoresis-development-consulting.de/html/semicolloidal.html

Hot Coomassie Staining Protocol

This hot Coomassie-staining is staining and fixing simultanuously, (Figure 14).
The acetic acid for staining and destaining can be of technical quality.

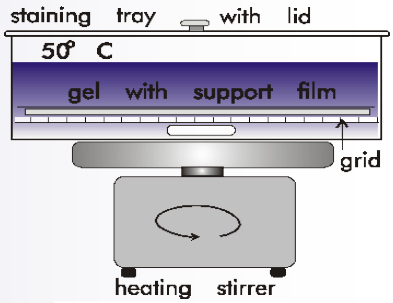


Fig.14: *Hot Coomassie -staining using the „Staining Tray Normal"*

Stock solutions:

staining solution:	0.03 % (w/v) Coomassie R-350 in 1250 mL 12.5 % acetic acid
destaining solution:	12.5 % acetic acid
preserving solution:	10% (v/v) glycerol

If Pharmalytes were used in the first dimension this 2 washing steps should be done first:

Washing programm:	40% EtOH/10% HAc for 30 min
(*Pharmalytes in 1.dim.)*	20% EtOH/10% HAc for 30 min
Staining programme:	Staining: 2 h *fresh* staining solution at 60 °C (in a fume hood) while stirring (Fig. 14), stain the 15% gels 3 h.
	Destaining: Overnight in a small volume destaining solution in a tray - film-side down - on a rocking platform.
	Preserving: 10 min preserving solution
	Drying: Air-dry overnight

Silver Staining Protocol

Follow also: http://www.electrophoresis-development-consulting.de/html/2dsilver.html

For all steps:place gel onto the glass tray bottom, gel side up

step	reagent	volume	time
1 Fixing	40% (v/v) Ethanol / 10% Acetic Acid (v/v)/ 1% Citric Acid *(160 ml EtOH/40 ml HAc/4 g Citric Acid in 400 mL)*	2 x 400 mL	2 x 30 min
2-5 Washing	H_2O dist	4 x 200 mL	4 x 10 min
6 Sensiti-zing*	30% Ethanol; 0.03% (w/v); Sodium Thiosulphate; 10 mmol/l Sodium Acetate *(60 ml EtOH/65 mg Thiosulfate/300 mg NaAc in 200 mL)*	200 mL	30 min
7-10 Washing	H_2O dist	4 × 200 mL	4 × 5 min
11 Silvering	0.25% $AgNO_3$ / 0.03% Formaldehyde (w/v) *(500 mg $AgNO_3$/180 µl Formaldehyde (37%) in 200 mL)*	200 mL	20 min
12-14 Washing	H_2O dist	3 × 200 mL	3 × 1min
15 Developing	2.5 % Na_2CO_3 / 0.03% Formaldehyde/ 0.00075% Sodium Thiosulfat *(5 g $NaCO_3$/180 µl Formaldehyde/ 75 µl Sodium-Thiosulfat (2%) in 200 mL)*	200 mL visual con-trol!	3-5 min
10 Stopping/ Preserving	10% HAc, 10% Glycerol *(20 ml HAc/20 ml Glycerol in 200 ml)*	200 mL	20 min
11 Drying	air dry , on the support-film		16 h

* without Glutardialdehyde, because of the mass-spectrometer!

Fluorescence-Labeling T-REX Protocol (for Polyester film support)

From „ **T-REX 310 Protein Labeling Kit"**, Dyagnostics GmbH, Halle (Germany)
http://www.electrophoresis-development-consulting.de/html/downloads.html

Labeling protocol
In order to keep pipetting losses to a minimum we recommend to use low retention mass spec compatible pipette tips and microcentrifuge tubes (e.g. NH DyeAGNOSTICS product no. PR052).
1. Allow the T-REX 310 vial to warm up to ambient temperature (ca. 5 min).
2. Centrifuge briefly.
3. Dilute the dye in 2 µl T-REX 310 solvent. The solvent turns pink.
4. Mix (Vortex) and spin down briefly.
5. Transfer 50 µg of protein sample (ideally up to 10 µl, 20 µl maximum) into the vial.
6. If necessary, add sample buffer until a total volume of 12 µl.
7. Mix (Vortex) and spin down briefly.
8. Incubate the reaction mixture for 30 min on ice.
9. Stop the labeling reaction by adding of 2 µl of T-REX 310 labeling stop solution.
10. Mix (Vortex) and spin down briefly. Incubate the reaction mixture for 10 min on ice.
11. The protein sample is now labeled with T-REX 310, and ready to use for downstream applications.

Recommended sample buffer
Do not heat lysis buffer to dissolve urea. Store in small aliquots at -20°C.

compound	**concentration**	**quantity**
Tris	30 mM	0,18 g
Urea	7 M	21,00 g
Thiourea	2 M	7,60 g
CHAPS	4% (w/v)	2,00 g

add deionized water to a total volume of 50 ml; adjust pH to 8.5

Excitation and imaging detection properties

	exitation max. [nm]	emission max. [nm]
T-REX 310	648	663

Detection
Use the recommended filter settings of your imaging system for G-Dye300, Cy5 or Alexa 647.
In order to get the optimum fluorescence performance of T-REX 310 we recommend a pre-scan for each gel in order to determine the optimum exposure time of your camera or the optimum detection voltage of your scanner. The signal of the strongest protein bands or protein spots should be marginally **below** saturation.

Storage
Store the T-REX 310 containing vials protected from light and dry at -20°C to -80°C. Labeled proteins can be stored for up to three months at -80°C until further use.
Best before: see packaging

Fluorescence-Visualization Protocol (for **non-fluorescent** film support)

From „**Lava-Purple Protocol**" Serva, Heidelberg (Germany)
For Polyester use a dye working in the green, red or infrared channel.

Prefixing: 20% ethanol, 7% acetic acid
Add 14 mL acetic acid and 40 mL Ethanol to 145 mL MilliQ (or equivalent) water.

Solution 1: 15% ethanol, 1% citric acid
Add 150 mL ethanol to 850 mL MilliQ (or equivalent) water and add 10g citric acid (1 sachet part A). Mix until dissolved. pH should be between 2.1 and 2.4 (no need to check usually).
The gel may be fixed in solution 1 overnight with no negative effects.

Solution 2: 100 mM sodium borate pH 10.9
Dissolve 6.2 g boric acid (1 sachet part B) and 3.84 g sodium hydroxide (part C) into 1 L MilliQ or similar
water. All reagents should be ACS grade or similar. pH should be between 10.8 and 11.2 (no need to check).

Solution 3: 15% ethanol
Add 150 mL ethanol to 850 mL MilliQ grade (or equivalent) water.

Staining solution:
Add 1 mL LavaPurple concentrate into 200 mL Solution 2.
NOTE: It is essential that the dye concentrate has been brought to room temperature and thoroughly mixed prior to being added. Removing concentrate from -20°C after the fixing steps will ensure the solution is ready for use. The solution must be made fresh (not more than 30 minutes prior to use).
The concentrate must be added to solution 2 before being poured onto the gel; if the concentrate is pipetted directly into the tray, staining artifacts will occur. It is not necessary to protect the gel from light. Do not stain longer than 3 hrs as signal will decrease after this time.

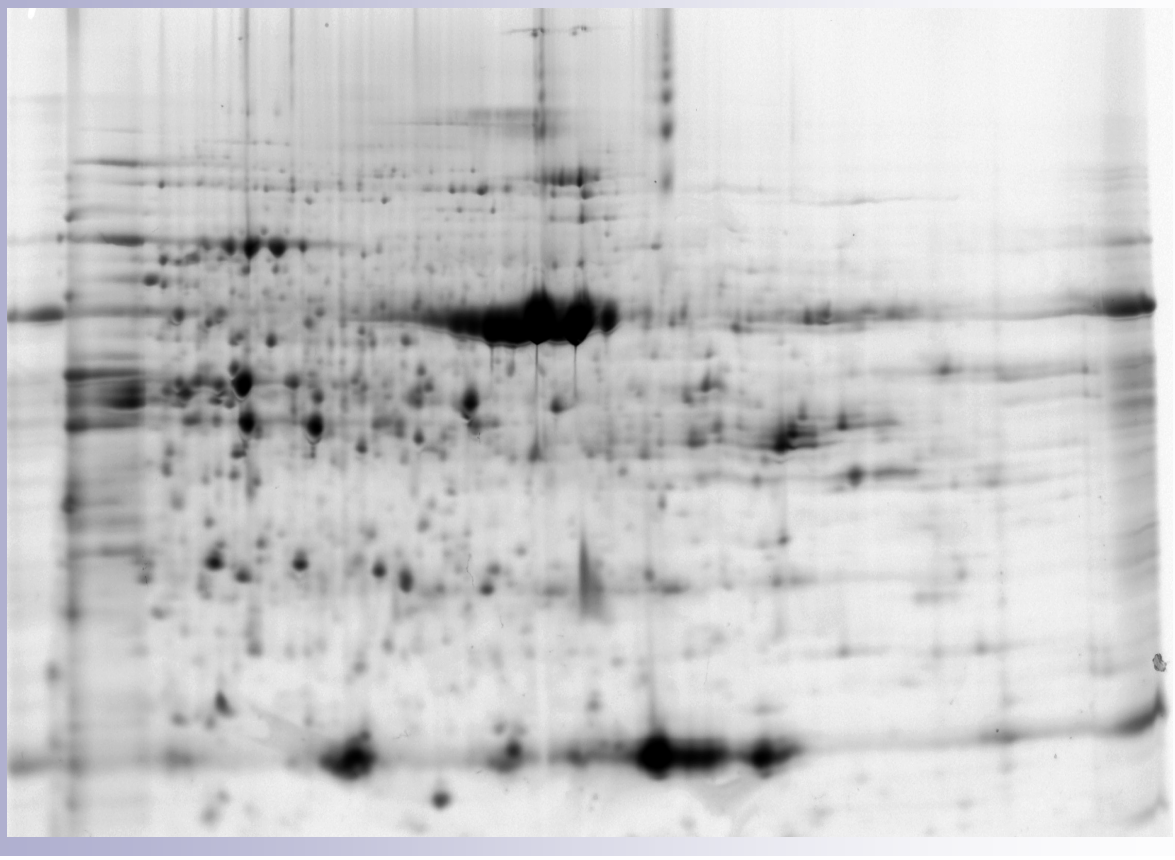
Acidification: Solution 1. If there is no time for scanning you can leave the gel in the acidifier solution (1) overnight.

Process	Recipe	Volume	Time
Prefixing	20% ethanol/7% HAc	200 mL	30 min
Fixing	Solution 1	200 mL	30 min
2 x Prebuffering	Solution 2	200 ml	2 x 15 min
Staining	Solution 2 + 0.5 % LavaPurple	200 mL	60 min
Washing	Solution 3	200 mL	30 min
Acidify	Solution 1	200 mL	30 min

Scanning:
The gels are scanned with the gel surface down facing the platen directly after applying a few mL water on it (focal plane 0). During scanning a *LF glass plate* or the new *Scan Frame* is laid on the gel to avoid curling of the edges. Do not apply „press sample"!
For accurate spot picking the scanning orientation of must be flipped as shown here:
Excitation maxima: 390, 500, Common excitation sources include: UVA together with 473 nm, 488 nm, and **532 nm** lasers. Emission maxima: **610 nm**. Common filters: 560 LP, 610 BP30, 600 BP10
Example: using Typhoon™: 532 nm laser, 540 PMT, 610BP30 filter, 100 µm resolution, normal sensitivity.

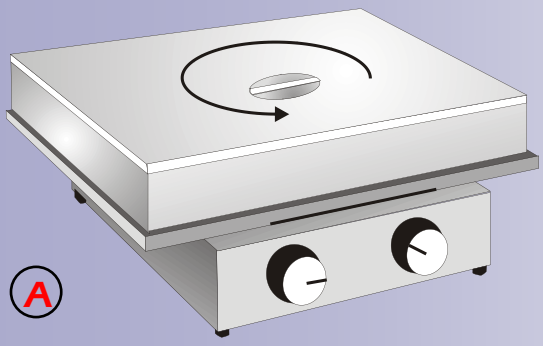
Comassie-Staining with NF-supported gels: Semi-Colloidal Coomassie at 40°C
http://www.electrophoresis-development-consulting.de/html/semicolloidal.html

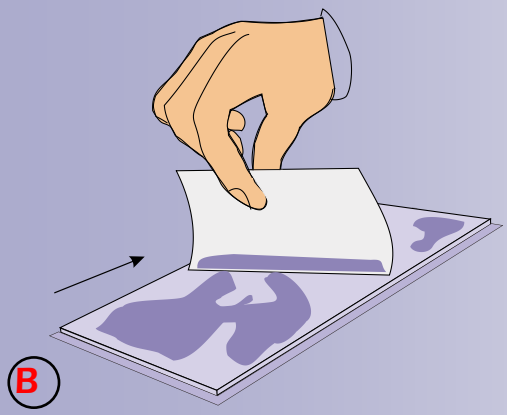
From „Brochure: Protein Labeling Kits:
Saturn-2D, Refaction-2D", Dyagnostics, Halle (Germany)
http://www.electrophoresis-development-consulting.de/html/downloads.html

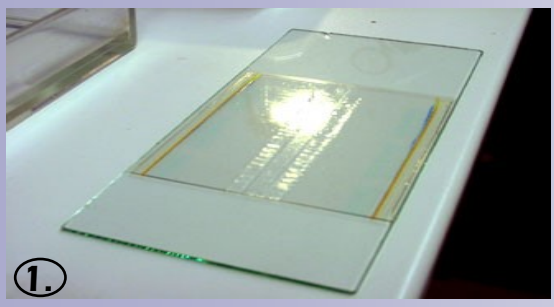


Blotting of 2D-Gels

From brochure: „Blotting of Horizonztal Gels“:
http://www.electrophoresis-development-consulting.de/blothor.pdf
Special selective visualizations, f.e. use of antibodies function best when the 2D-result is blotted on a blot-membrane. Easiest way to do this is the „Contact-Blot“, page #2 and 3 in the brochure.







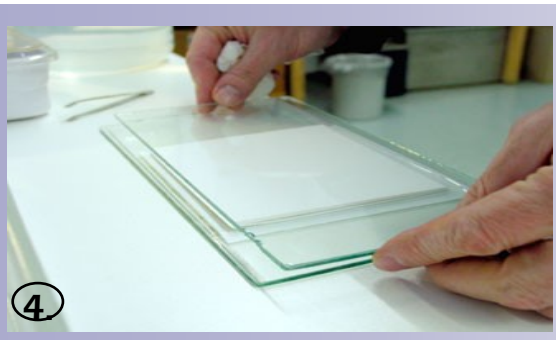
Contact Blot
The most simple blotting method after a SDS– or 2D electrophoresis is the “Contact Blot”. Using this transfer-method the plastic-support of the SDS-gel must not be removed.
Because water must pass the membrane, a hydrophilic material is recommended: Nitrocellulose, 0.2 µm pore size (page Alternatively: PVDF-membranes (Teflon) will work also.

Please note that SDS– and 2D-gels - run longer than 2 - hours - have to be equilibrated in a buffer solution to amend the loss of water during electrophoresis.

After the electrophoretic procedure the blotting membrane is directly layed on the gel-surface. With the help of dry filter paper and a weight the SDS-proteins are forced to migrate onto the membrane. For SDS-proteins run in a 12.5% T gel nice results are achieved up to 70 kD. Bigger proteins should be blotted overnight and/or run in a 7.5% gel.
This blotting method is no quantitative technique, but sufficient for many qualitative tests.
Cut the necessary filter papers (5 x Whatman´s No.1) and the blotting membrane to the size of the gel (25.5 cm x 11.5 cm).

⇒ A) *The gel should be first equilibrated 30 min in 50 mM Tris/Cl pH 8.0, 0.05% SDS to amend the gel's lost water content.*
B) *Then dry the gel's surface with a Drying Cardboard.*

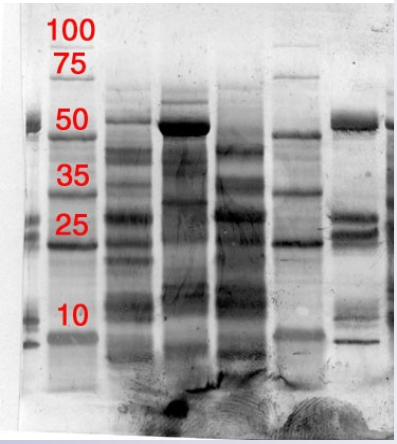
⇒ *The PVDV-membrane is activated in 100% methanol for 10 minutes. This step is not necessary for Nitrocellulose-membranes. The membrane is then placed in dist. Water for 5 min.*
⇒ *The SDS-gel is placed on a glas-plate with the support-film down. Fig.1.*
⇒ *The membrane is layed on the gel-surface, avoid air-bubbles! Fig.2.*
⇒ *Five dry and clean filter-papers are layed onto the membrane*

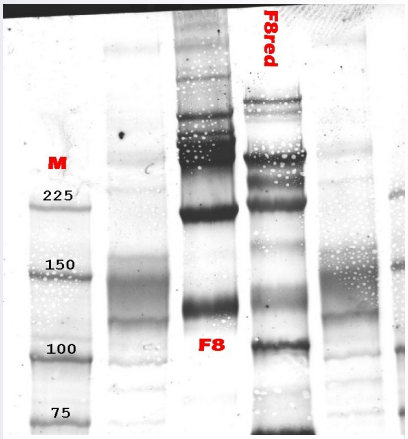


⇒ An additional glas-plate is applied on the stack. Fig.4.
⇒ A 2-3 kg weight is standing on the contact-blot for 2 hours (IEF) and 4h – overnight for SDS– 2D- and native-gels. Fig. 5.

Staining: Indian Ink see below and also page 9.

Contactblot out of a 12.5 % T gel, transfer: 5-75 kD





Contactblot out of a 7.5 % T gel, transfer up to 800 kD