SDS Electrophoresis with ElphoGel Kit SDS *EQ-type*

V18 05/23

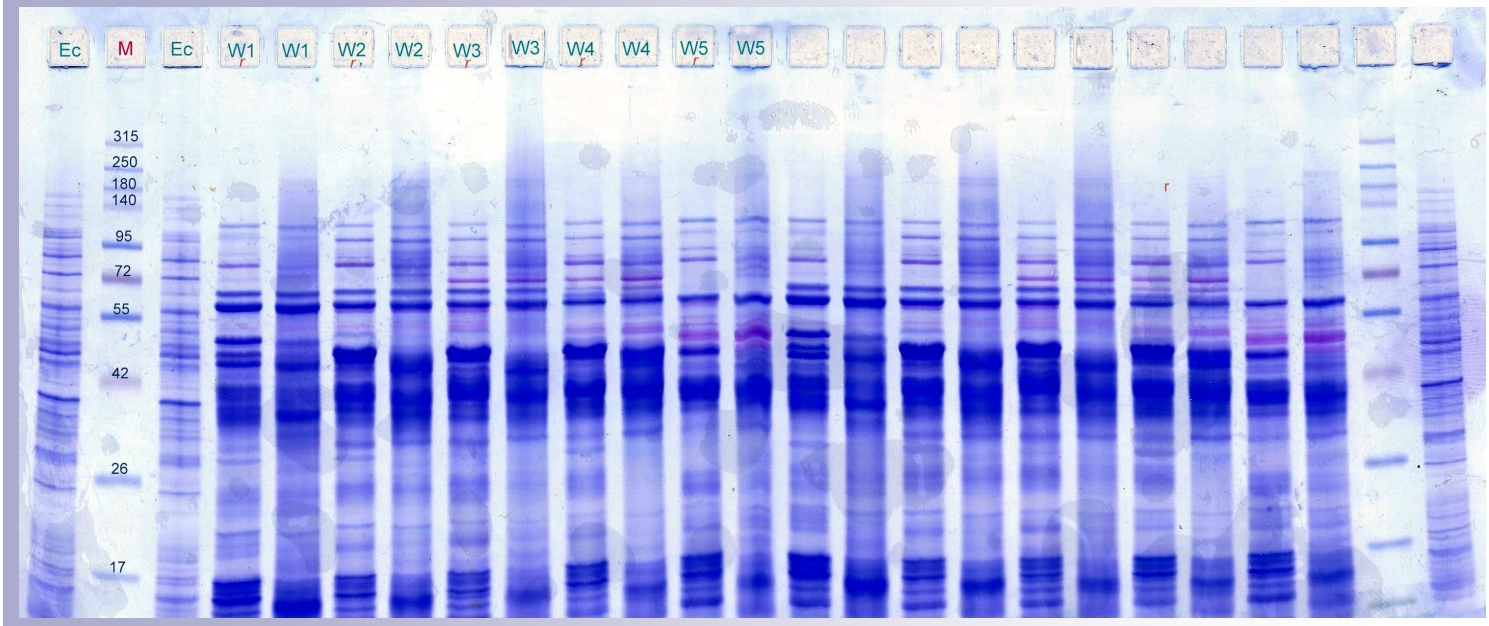


Fig.1: *Different wheat varieties on ElphoGel SDS 12.5% 25S EQ-type. Hot Coomassie stained.*

***General**: „ElphoGel SDS" are designed for horizontal electrophoresis.*
A special buffer-system was developed to give best resolution.
New types of matrices were designed to get the same quality as in vertical electrophoresis methods.
The mostly used electrophoretic method for proteins is the SDS-electrophoresis. Because here the migration speed of the applied protein-SDS-mycells is relative to the molecular-weight. All proteins are running in the same direction (to the anode), and also the lipophylic proteins are soluble as SDS-mycells.

In vertical-systems the Laemmli -system (1,2) is used but the gel-buffer pH-value is very high (8.9 pH). To perform the electrophoretic separation at a neutral pH-value, a special SDS Buffer Kit Neutral was developed.

The electrode buffers are now delivered as gel-electrodes to improve both: the electrophoresis itself and the silver staining.

ElphoGels SDS EQ-type:
A new type of gel production lead to this new matrix: Best resolution and super sensitive silver-staining.

Polyester-support:
Using the normal polyester support film a „Hot Coomassie" staining (Fig.1), silver-stainings and fluorescent visualizations in the green, red and infra-red channel can be performed (Fig.2).
NF-support:
Non-fluorescent support (NF). These gels should be used for fluorescence-techniques when all 3 channels are needed, fig.1 right.
The fact that these gels can be scanned without any surrounding glass-cassette yields in higher sensitivity.

The Ready-To-Use ElphoGels can be stored at 4°/8°C for 6 month.

The following equipment is recommended:

Electrophoretic Hardware:

flatbed IEF professional	(edc-prof-2836)
Multiphor (discontinued)	(GE-18-1018-06)

Consumables:

ElphoGel SDS Kit 7.5% 25S *EQ-type*	(edc-4210)
ElphoGel SDS Kit 12.5% 25S *EQ-type*	(edc-4202)
ElphoGel SDS Kit 12.5% 52S *EQ-type*	(edc-4203)
ElphoGel SDS Kit 12.5% no slots *EQ-type*	(edc-4209)
ElphoGel SDS Kit 12.5% 25S NF *EQ-type*	(edc-4302)
ElphoGel SDS Kit 12.5% 52S NF *EQ-type*	(edc-4302)

including:
4 gels, 4 x 2 gel-electrodes, sample buffer, cooling fluid
only for DryGel SDS Kits: gel buffer 200 ml, drying cardboards

SDS 1D Gel Electrodes Kit (without gels)	(edc-5013)
10X SDS Sample Buffer (10-fold concentrated)	(edc-4201)
10X SDS Sample Buffer NF (10-fold concentrated for fluorescence)	(edc-4200)

Additionally necessary:

DryPool Combi (only for DryGel SDS Kits)	(*edc-me-d*)

Tray for rehydration of dry gels (normal size) and soaking electrode strips

For SDSGels without slots:
The stacking gel (cathodal side) is opposite the round corner!

Marking the SDS 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 with the samples and for orientating while processing IPG-strips in 2D electrophoresis.

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

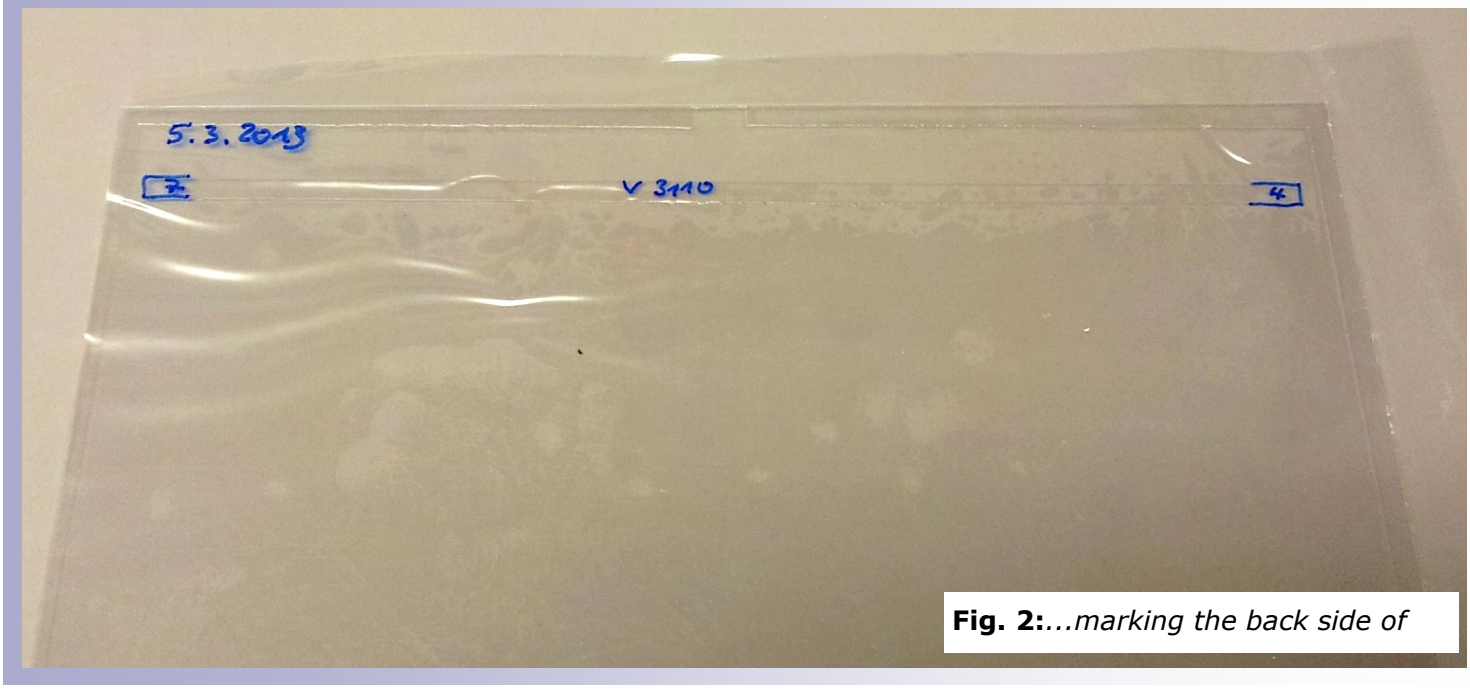


Fig. 2:*...marking the back side of*

Sample preparation

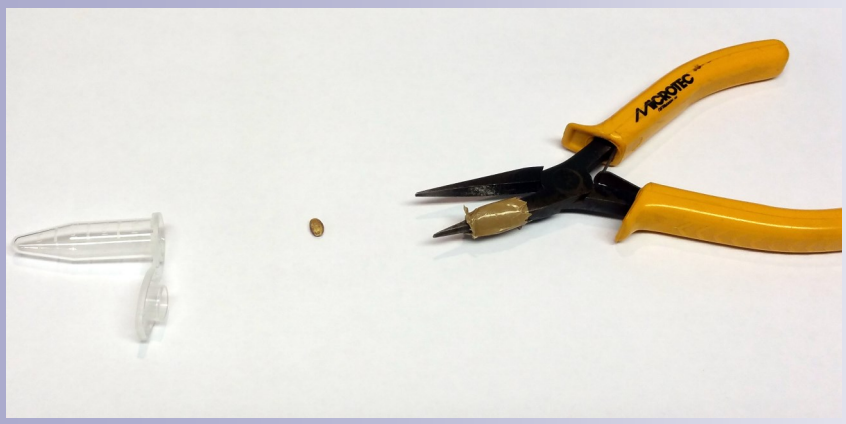
Sample buffer: 15 ml rehydration buffer + 10 ml H_2O bidest + 500 mg SDS + 80 µl Orange G solution (1%) + 60 µl bromophenol blue solution (1%)
Sample extraction: Extraction Buffer: 250 mM TRIS + 190 mM Glycine + 1 mM EDTA, gives pH of 9.2
10 mg E.coli + 1 mL Extraction Buffer + 180 mg Urea (3M, freshly added!) 15 min Ultrasonic treatment, centrifuge 5 min.

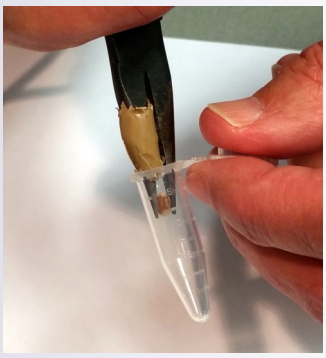
Sample treatment: Dilute the samples with the sample buffer at least 1 + 1. Dilute as much as possible,to reach the upper nonogramm region, this gives best results with Coomassie.
To control the samples concentrations: Take Lonza´s "Molecular Weight" standard 5 - 225 kD (Lonza 50547) add the same volume sample buffer and run at least one lane per gel.
After staining procedure, the samples should appear in the same state than this standard lane. Apply 13 (25S)-5 µl (52S) of each sample, don´t leave sample slots unfilled.
10X Sample Buffer: For diluted samples! Dilute 90 µl sample with only 10 µl SDS-buffer.

After the samples dilution is done add 5% (v/v) DTT-solution (1% w/v) to the vials (reduction!) and heat 10 min at 70°C. After the vials are cooled down add 5% (v/v) IAA solution (4% w/v) to the samples (alkylation!).

Special Sample Preparation for Wheat, Barley.

Extraction Buffer: 0,194 g Di Potassiumhydrogenphospate, 0,528 g Potassiumdihydrogenphosphate, 0,38 g EDTA, 1 g Dithiothreitol in 1000 ml.
Short before use: 7 ml Extraction Buffer + 400 µl Tetramethyurea (TMU = ~6% v/v) + 15 µl Bromophenolblue (1% w/v) + 100 µl ~Chloroethanol.
150 µl per one crushed and pulverized grain. 15 min ultra sonic treatment. Centrifuge the Eppendorfs 5 min 5000 rpm.
Then take 80 µl extracted volume + 350 µl Sample Buffer (incl. DTT) and heat 3 min 95°C.





cracking the grain

…and grating

Fig.3a– c: *radio pliers for cracking the grain. Philipps-screwdrewer for pulverising. One side with tape: this prevents splinters splashing out.*

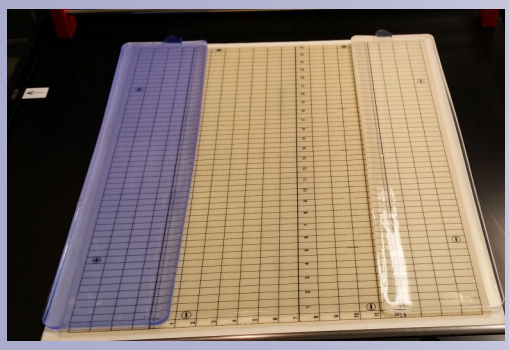


Fig.4: *flatbed horizontal chamber. Arrangement of gel and electrode strips on the cooling plate.*

Application of gel and gel-electrodes

Switch on the kryostate, adjusted to 15°C. Apply a very thin layer of the cooling fluid (ca. 1.5 ml) onto the cooling plate with a pipette, in order to ensure good cooling contact. Place the gel (surface up) on to the center of the cooling plate: The edge containing the wells must be orientated towards the cathode (Fig. 4).

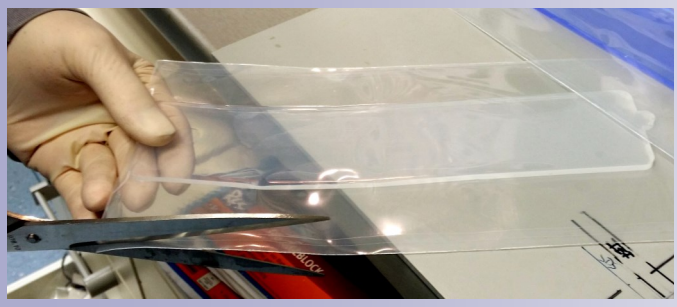


Fig. 5: *Cutting out the gel-electrodes out of their bags.*

Use gloves and clean forceps!

Cut out the gel-electrodes out of their bags, fig 5.

Carefully grap the gel-electrode and put them onto the gels with an overlapping zone of ~3-4 mm. Fig. 7.

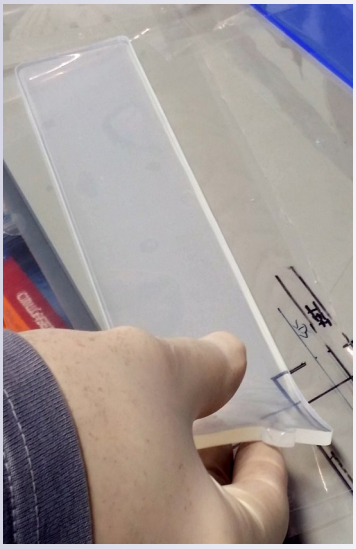


Fig.6 *Grap the gel-electrode….*

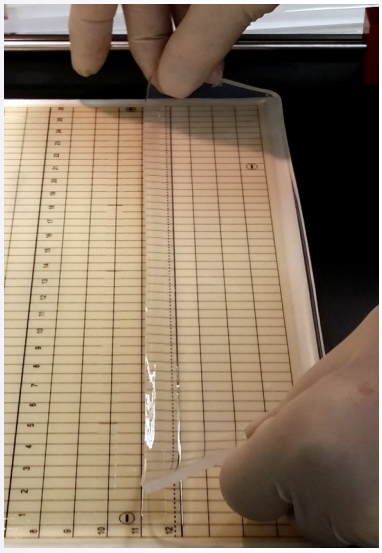


Fig.7: *…and place it onto the gel's edge*

Sample application and electrophoresis

Apply 5, res. 13 µl of each sample to the sample wells using a micropipette (or use appropriate multipipette). Don´t leave sample slots unfilled. Clean platinum electrode wires before (and after) each electrophoresis run with a paper.

flatbed basic and Multiphor: Place the security lid onto its 4 stands of the basic unit. Move the electrodes so that they will rest on the outer edges of the electrodes (fig. 8,9). Move the sliders forward and lower the safety lid until the platinwires stand on the electrodes.

flatbed IEF professional: Set the electrodes on their connectors with the apropriate electric orientation, pressing them down a little. Fig.9.

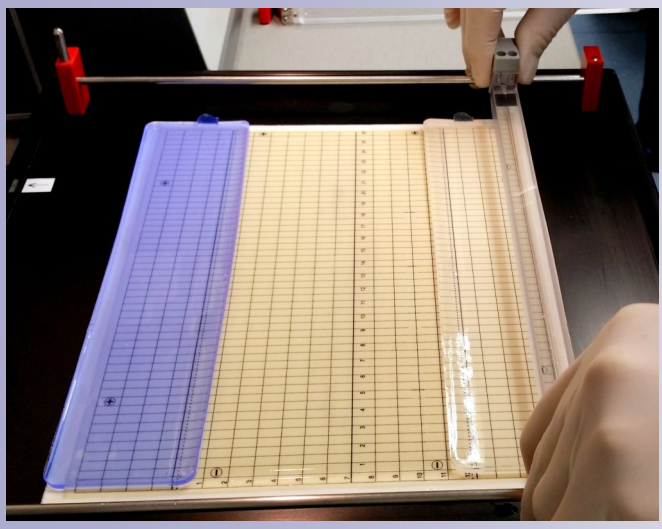


Fig.9: *flatbed IEF professional: Setting the platinum electrodes*

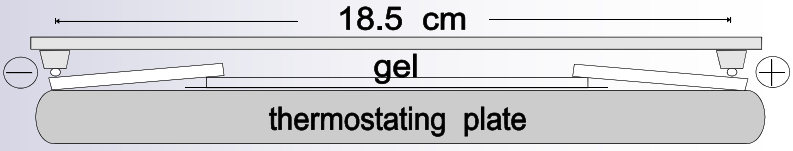
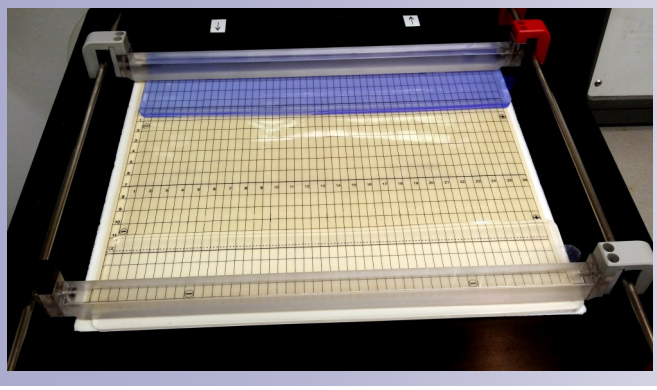


Fig.8: *Arranging of gel and electrode buffer strips*



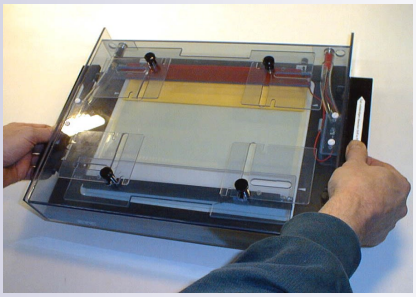


Fig.10: old flatbed horizontal chamber: Lowering the security lid

Running conditions (15°C):
Power supply settings for *normal proteins* (all ElphoGels SDS normal size)

Total: 2h *(Select „BASIC" mode)*

Bio-Rad Power Pac HV:
type in this parameter as constant —-> n mA
type in the other settings as limits —-> n V

1 Gel: half gel: half mA , half W	**Set V**	***Start Value***	**SET mA**	**Set W**	**Time**	**Comment**
Phase 1	250 V	*(~130 V)*	20 mA	10 W	30 min	sample entrance
Phase 2	500 V	*(~290 V)*	30 mA	15 W	90 - 120 min*	Stopping the elctrophoresis: see below

Settings for *complicated, unknown proteins and cereal samples (wheat, barley)*
Total: 2h 40 min, 25°C - 15°C

1 Gel: half gel: half mA , half W	**Set V**	***Start Value***	**SET mA**	**Set W**	**Temp (C)**	**Time**	**Comment**
Phase 1	100 V	*(~60 V)*	12 mA	5 W	25°C*	20 min	slow sample entrance #1
Phase 2	200 V	*(~120 V)*	20 mA	10 W	15° (set kryostat!)	20 min	slow sample entrance #2
Phase 3	250 V	*(~170 V)*	25 mA	10 W	15°	20 min	sample concentration
Phase 4	450 V	*(~240 V)*	30 mA	15 W	15°	120 min*	Stopping the elctrophoresis: see below

*Phase 1 and 2 serves as protein-entering: 25°C in the first step.
Do not forget to lower the kryostat-setting in phase 2.
For the 7,5%T gels the starting Volt-values are a little bit lower.
*Stopping the electrophoresis:
Run time is variable due to different %T-values and salt loading of the samples.
Stopping by time and Vh, or better control with the red Conchenille, supplied with the sample buffer: When the red dye (Conchenille (CON), behind the SDS-zone) is just before the anodal strip.
This means: the Bromophenolblue (BPB) **incl. the SDS-zone** have to leave the gel!, see below.
The run should then be stopped and the gels must be placed immediately into the fixing solution.
See page #5 and #6.

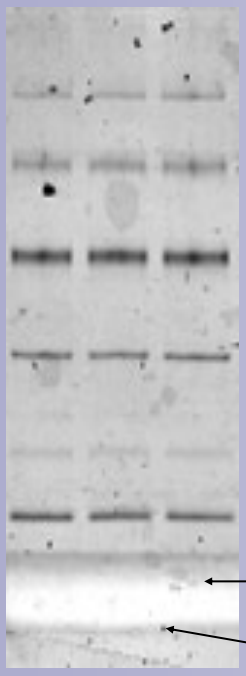
Optimal shutdown
The (andodal ion) SDS should be completely in the anodal strip!
In EDC's SDS buffer-system the SDS runs behind the Bromophenolblue.
This means: Stopping the run with the BPB will left ~1.5 cm SDS in the separation gel.
Remedy:
Let the SDS run out of the gel and use the separation distance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

See next page

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.

See the images next page….



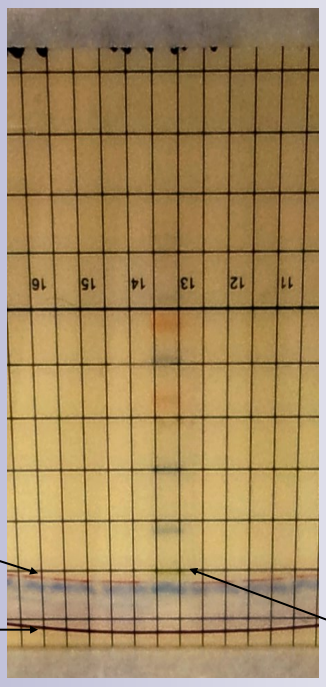
17 kDa

10 kDa

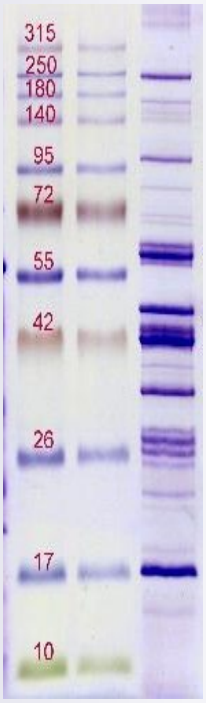
CON

SDS

BPB



run



SDS is in the anodal strip and the 10 kDa is just before the strip

SDS-zone is still in the gel

Detection of protein bands

1. Hot Coomassie R-350 staining:

This hot Coomassie-staining is staining and fixing simultanuously!
The acetic acid for staining and destaining can be of technical quality.

Stock solutions:

staining solution: 0.03 % (w/v) Coomassie R-350 (GE 17-0518-01),
1 tablet (corresponds to 0.4 g dye substance) in 1250 ml 12.5 % acetic acid.
destaining solution: 12.5 % acetic acid
impregnating solution: 10% (v/v) glycerol

Staining programme:

30 min *fresh* staining solution at 50 - 60 °C (exhauster) while stirring, fig. 11).
3 x 20 min destaining solution in a tray on a rocking platform, see fig.12*
20 min impregnating solution (tray), figure 12.
roll on the preserving sheet

*Optimal staining: can be achieved when the gel is placed in the first destaining solution overnight at ambient temperature

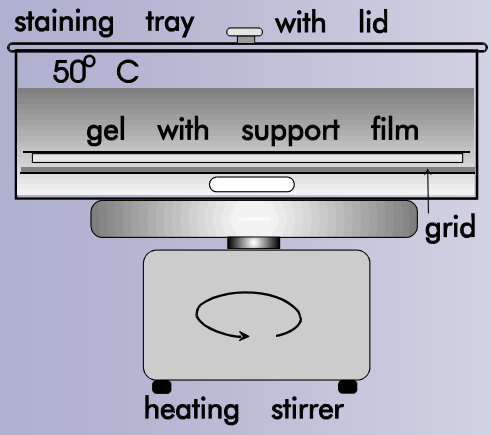


Fig.11: Hot Coomassie-staining

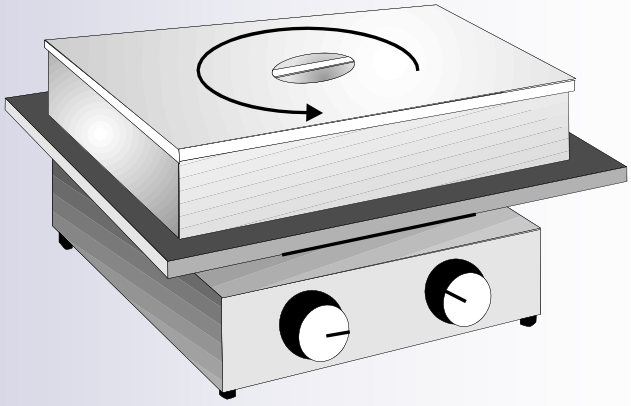


Fig.12: Destaining in a tray

2. Silver-staining:
Should be 50 times more sensitive than the Coomassie staining.
Recipe: http://www.electrophoresis-development-consulting.de/html/edcsdsag.html

This is a special silver staining recipe: EDC's gels have different buffer molecules and therefor they will need another staining procedure!

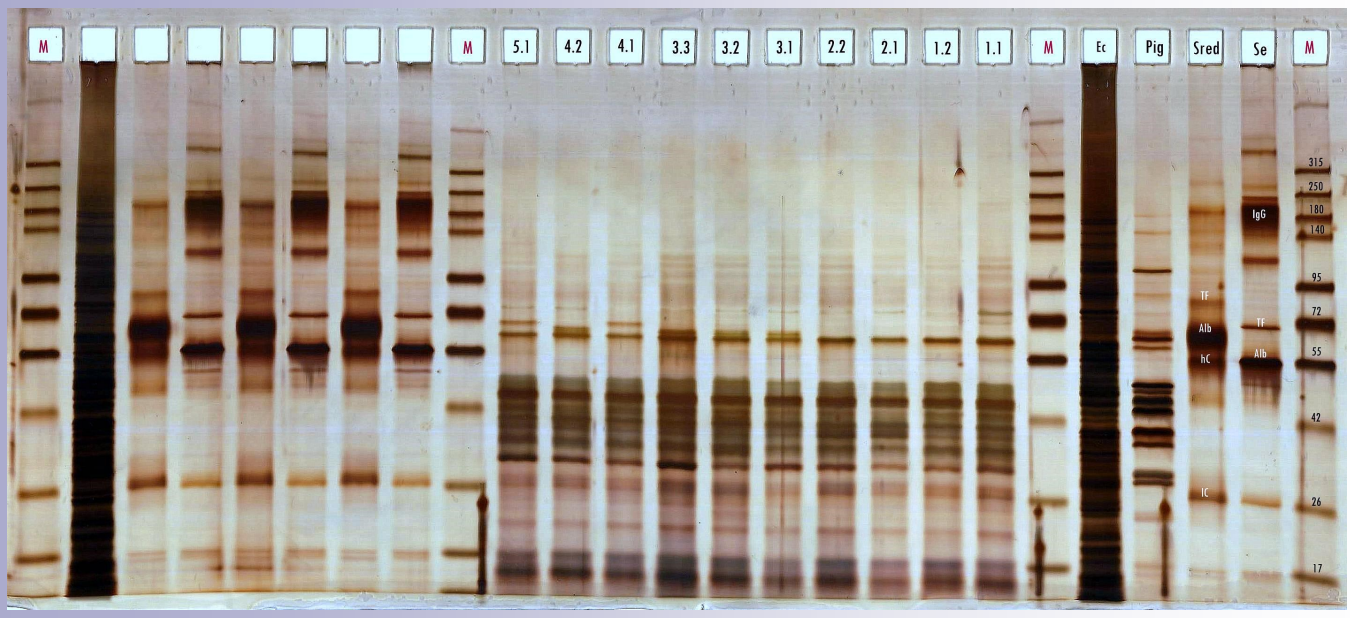


Fig.13: Silver-staining

3. Fluorescence-staining:
Should be 100 times more sensitive than the Coomassie staining.
Using the normal polyester support-film the green, red and infrared channel can be use.

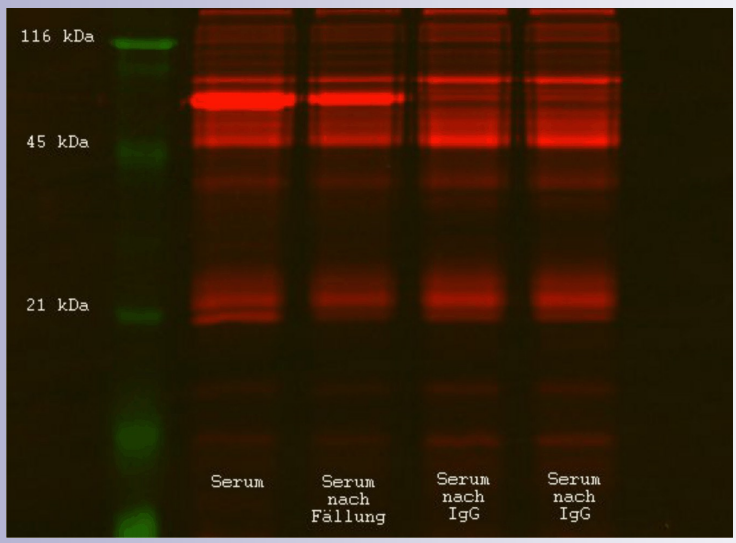


Fig.14: Proteins were prelabeled with T-Rex in the red and green channel

Fig.15: SDS-standard and different protein samples

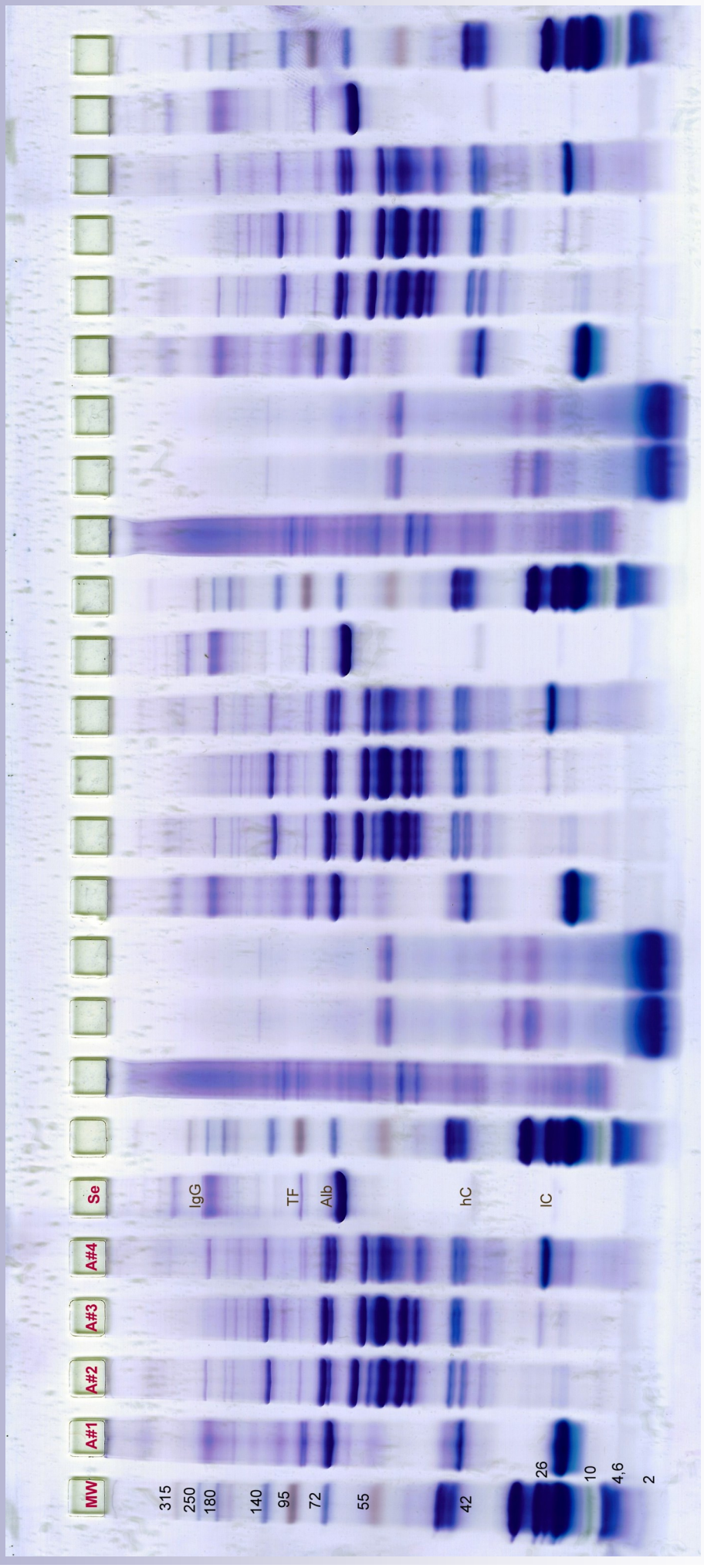


Fig.16: Five different wheat varieties on ElphoGel SDS 12.5% 25S *EQ-type*

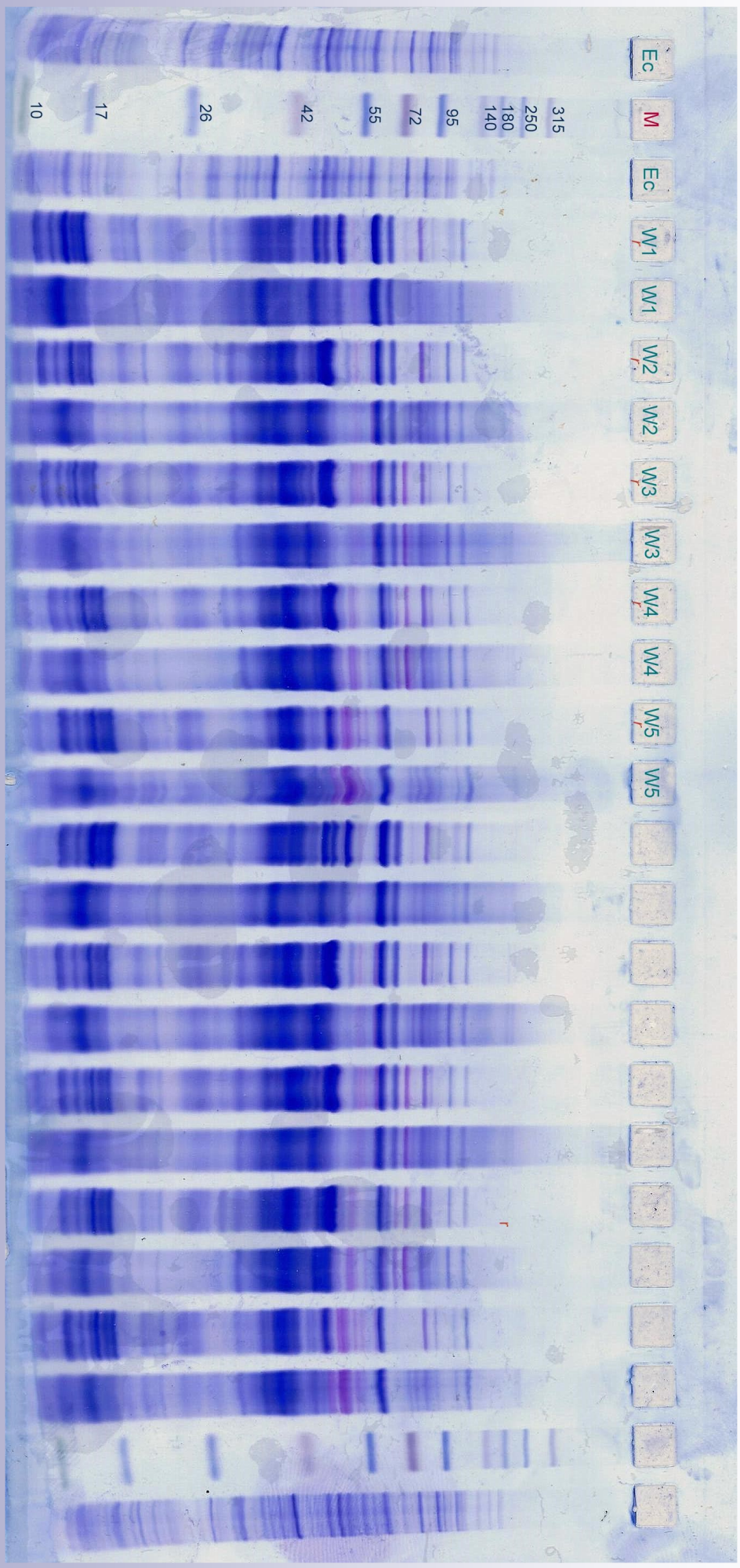


Fig.17: Different samples on ElphoGel SDS 7,5%25S *EQ-type*