SDS Electrophoresis with ElphoGel Kits SDS *short*

EDC V02 02/20

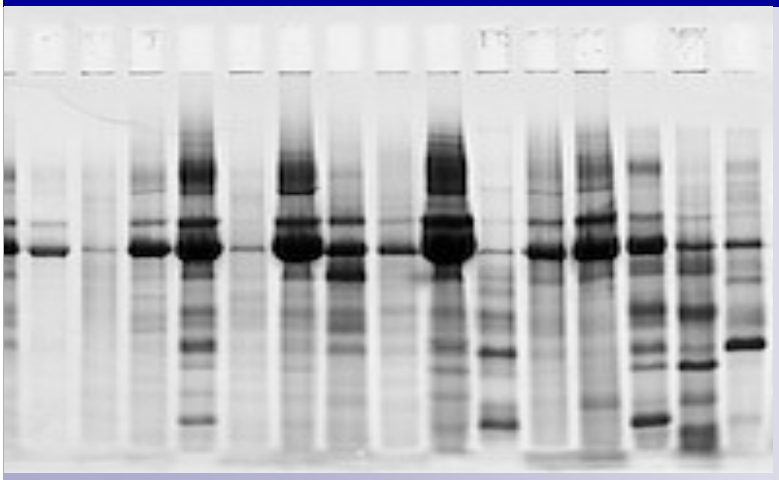

Fig.1: *Urine proteins on 12.5% 25S short. Fluorescence marked, red channel.*

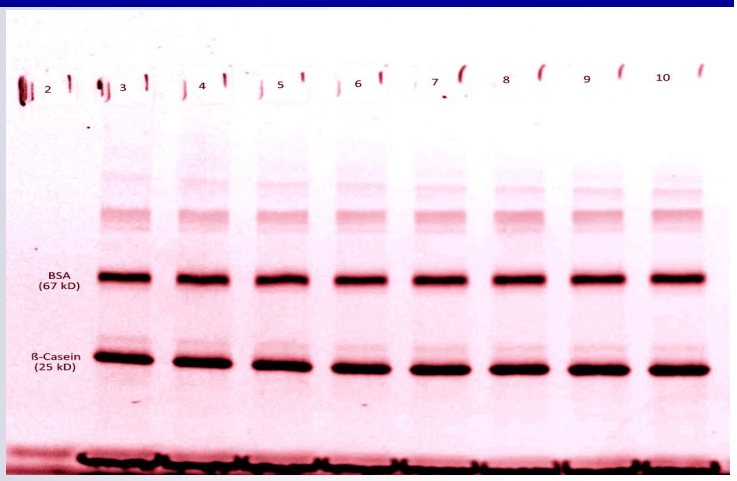

Fig.2: *Standards on 12.5% 25S short NF. Fluorescence marked, blue channel.*

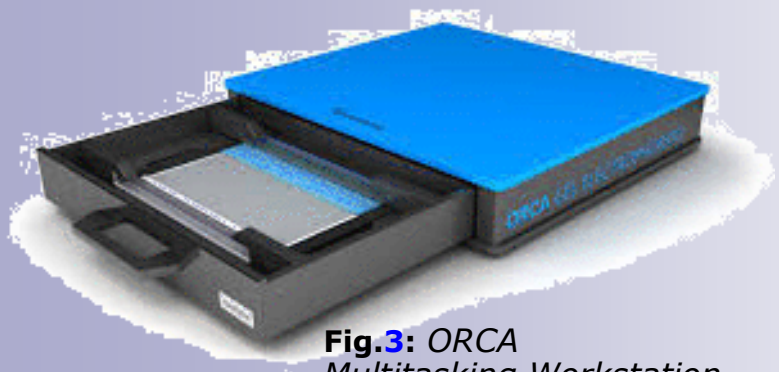

Fig.3: *ORCA Multitasking Workstation*

Fig.4: DOLPHIN Gel Electrophoresis

***General**: „ElphoGel SDS short" are designed for horizontal electrophoresis on Dyeagnostics ORCA Multitasking Workstation (fig. 3) or DOLPHIN Gel Electrophoresis (fig.4). Separation distance is 8 cm. Run time is ~ 80 min.*

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.

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
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 following equipment is recommended:

Electrophoretic Hardware:

ORCA Multitasking Workstation	(dyeagnostics PR204)
DOLPHIN Gel Electrophoresis	(dyeagnostics PR211)

Consumables:

ElphoGel SDS Kit 12.5% 25S	(edc-4211)
ElphoGel SDS Kit 12.5% 52S	(edc-4212)
ElphoGel SDS Kit 12.5% 25S NF	(edc-4311)
ElphoGel SDS Kit 12.5% 52S NF	(edc-4312)

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

10X SDS Sample Buffer (10-fold concentrated)	(edc-4201)
10X SDS Sample Buffer NF (10-fold concentrated for fluorescence)	(edc-4200)

Sample preparation for staining (after the run)

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!).

Sample preparation for fluorescence marking (before the run)

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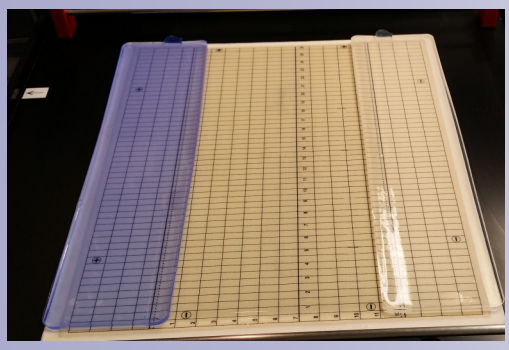


Fig.5: *flatbed horizontal chamber. Arrangement of gel and electrode strips on the cooling plate.*

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. 5).

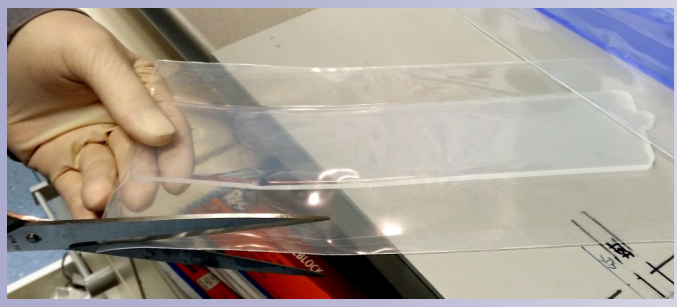


Fig. 6: *Cutting out the gel-electrodes out of their bags.*

Use gloves and clean forceps!

Cut out the gel-electrodes out of their bags, fig 6.

Carefully grap the gel-electrode and put them onto the gels with an overlapping zone of ~3-4 mm. Fig. 8.

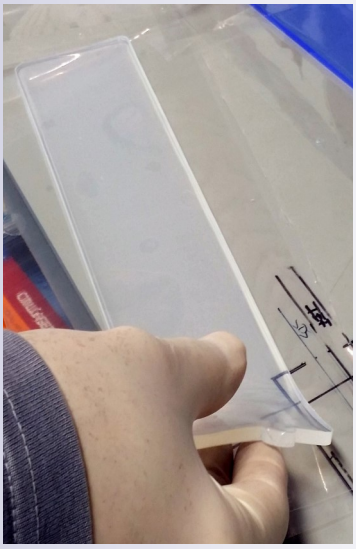


Fig.7 *Grap the gel-electrode….*

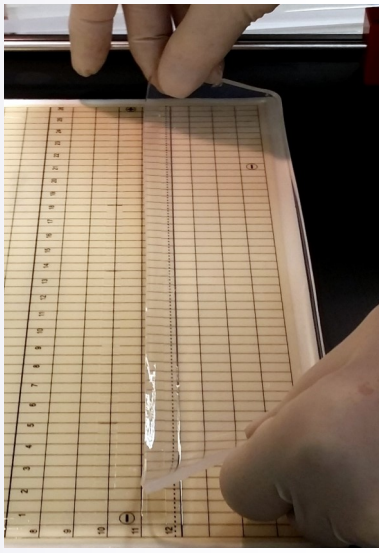


Fig.8: *…and place it onto the gel's edge*

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. 9,10). 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.11.

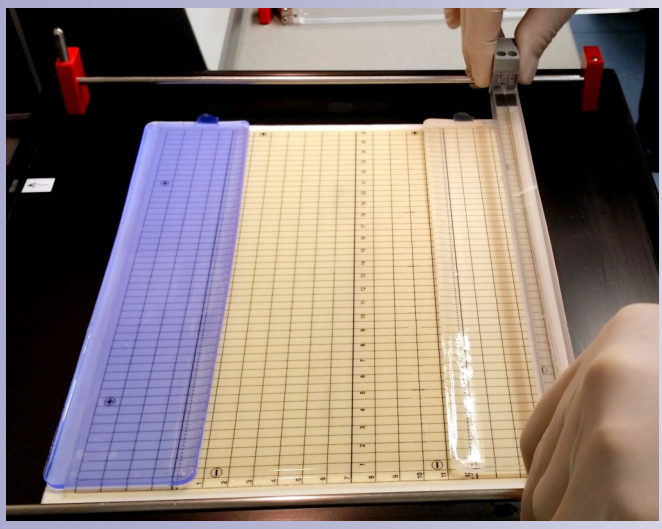
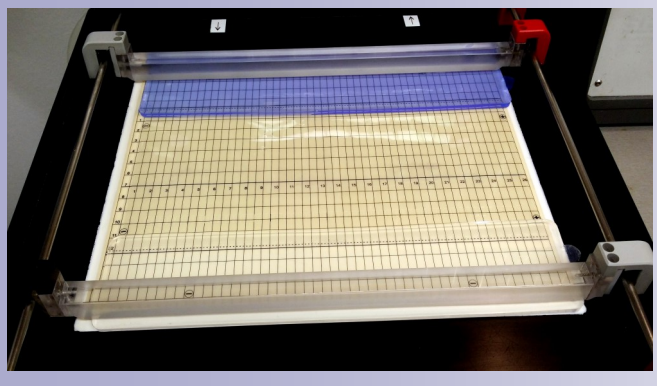


Fig.9: *flatbed IEF professional: Setting the platinum electrodes*



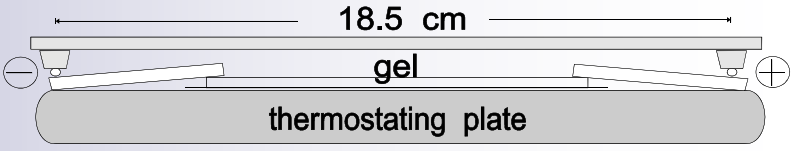


Fig.10: *Arranging of gel and electrode buffer strips*

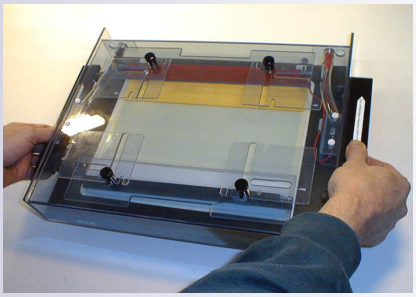


Fig.11: old flatbed horizontal chamber: Lowering the security lid

Running conditions (15°C):
Power supply settings for *normal proteins* (all ElphoGels SDS short) 80 min

Normal electrophoresis (~90 min)

1 Gel:	Set V	*Start Value*	SET mA	Set W	Time	Comment
phase 1 (sample entrance 1)	300 V	*(170 V)*	30 mA	10 W	25 min	buffer discontinuity through the slot
phase 2 (sample concentration)	500 V	*(280 V)*	42 mA	20 W	20 min	buffer discontinuity in the stacking gel
phase 3 (main electrophoresis)	800 V	*(460 V)*	60 mA	50 W	45 min	buffer discontinuity in the resolving gel

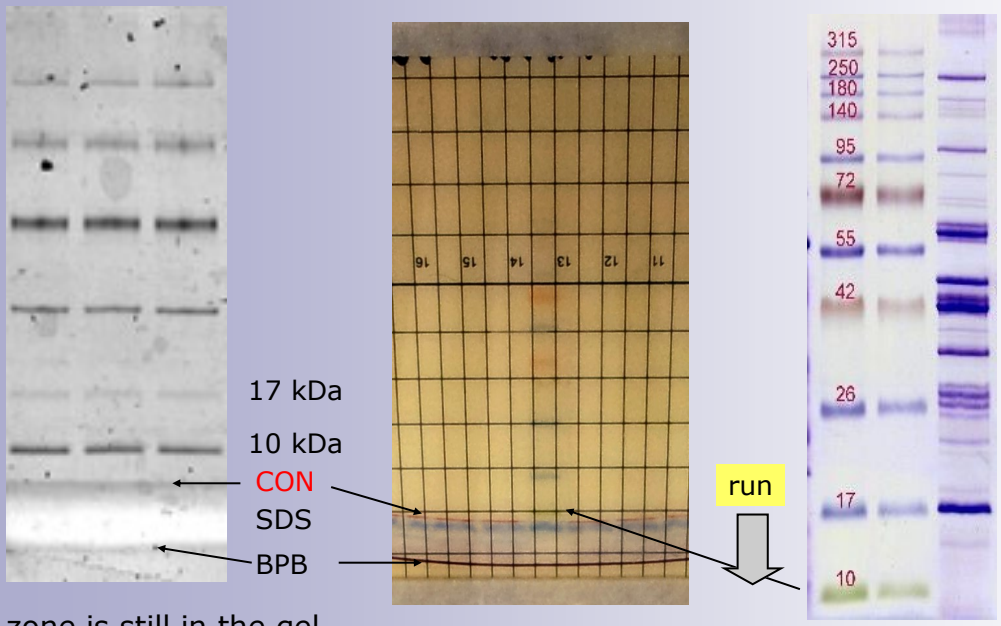
Bidirectional electrophoresis (~90 min)

1 Gel:	Set V	*Start Value*	SET mA	Set W	Time	Comment
phase 1 (sample entrance 1)	300 V	*(140 V)*	60 mA	20 W	25 min	buffer discontinuity through the slat
phase 2 (sample concentration)	500 V	*(250 V)*	85 mA	30 W	20 min	buffer discontinuity in the stacking gel
phase 3 (main electrophoresis)	650 V	*(400 V)*	100 mA	60 W	45 min	buffer discontinuity in the resolving gel

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

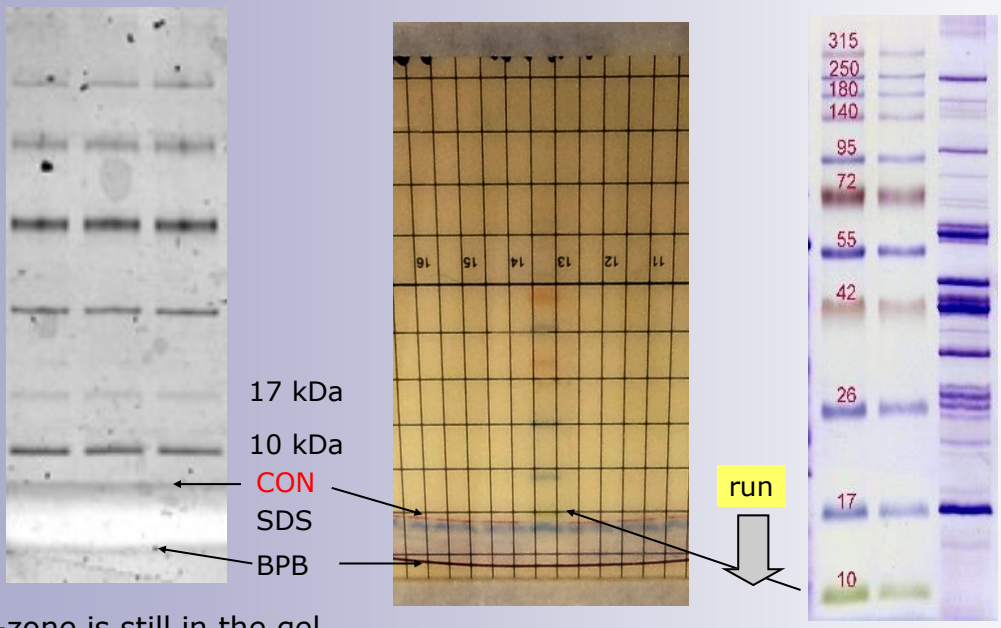
You can see the backside of the SDS-zone, marked red by the Conchenille dye from EDC's Sample Buffer. Let this zone out in the anodal strip, then shutdown.



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

SDS-zone is still in the gel

b) Use a prestained standard and run it till the 10 kDa stays just before the anodal strip.



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. 12).
3 x 20 min destaining solution in a tray on a rocking platform, see fig.13*
20 min impregnating solution (tray), figure 13.
roll on the preserving sheet

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

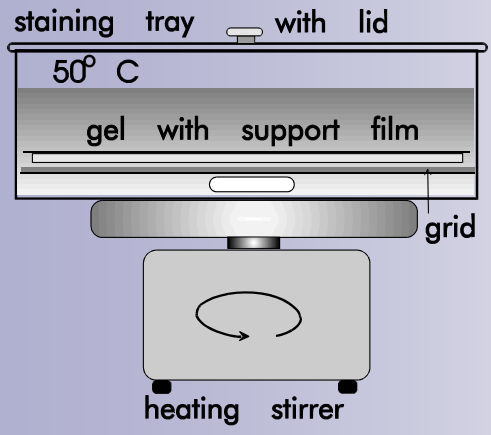


Fig.12: Hot Coomassie-staining

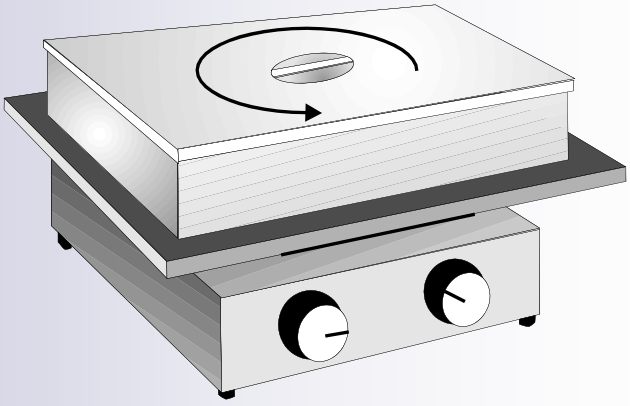


Fig.13: Destaining in a tray

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!

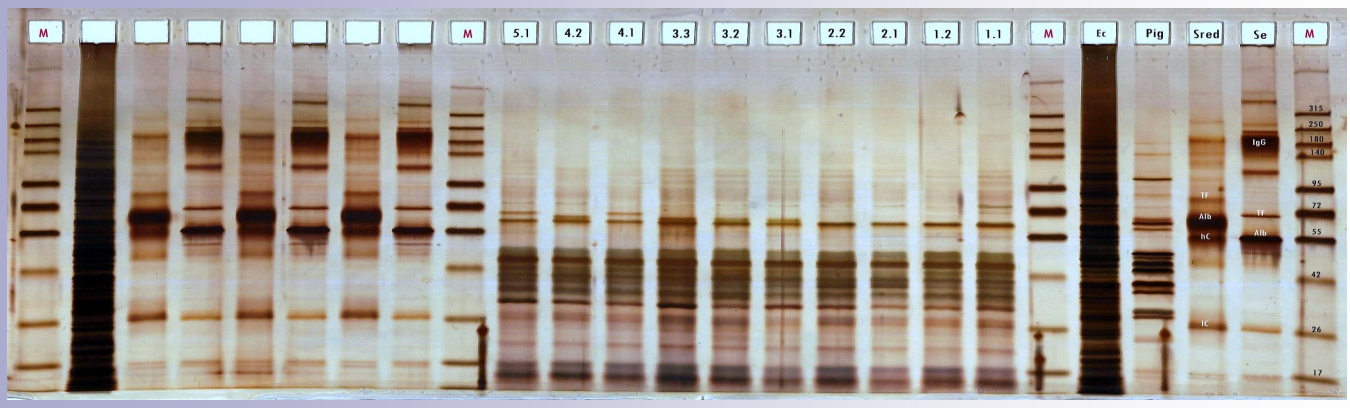


Fig.14: Silver-staining

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.

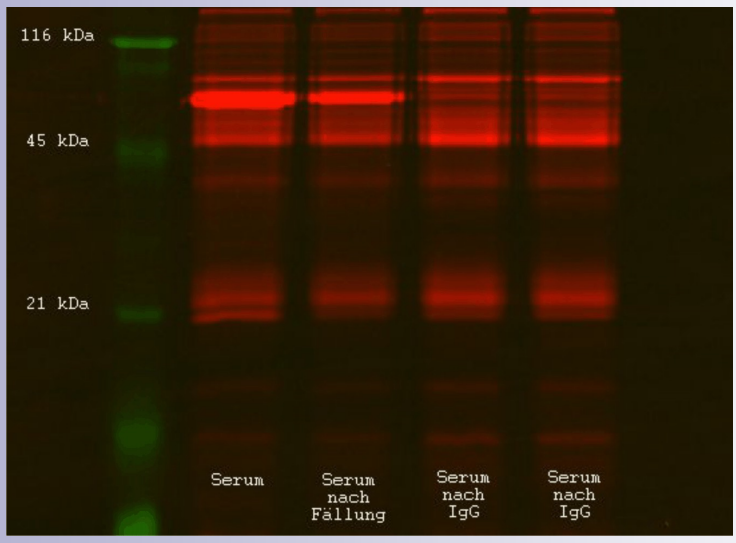


Fig.14: Proteins were prelabeled with T-Rex in the red and green channel