Electrophoresis of Tomato Leaf Extracts

EDC V1 05/14

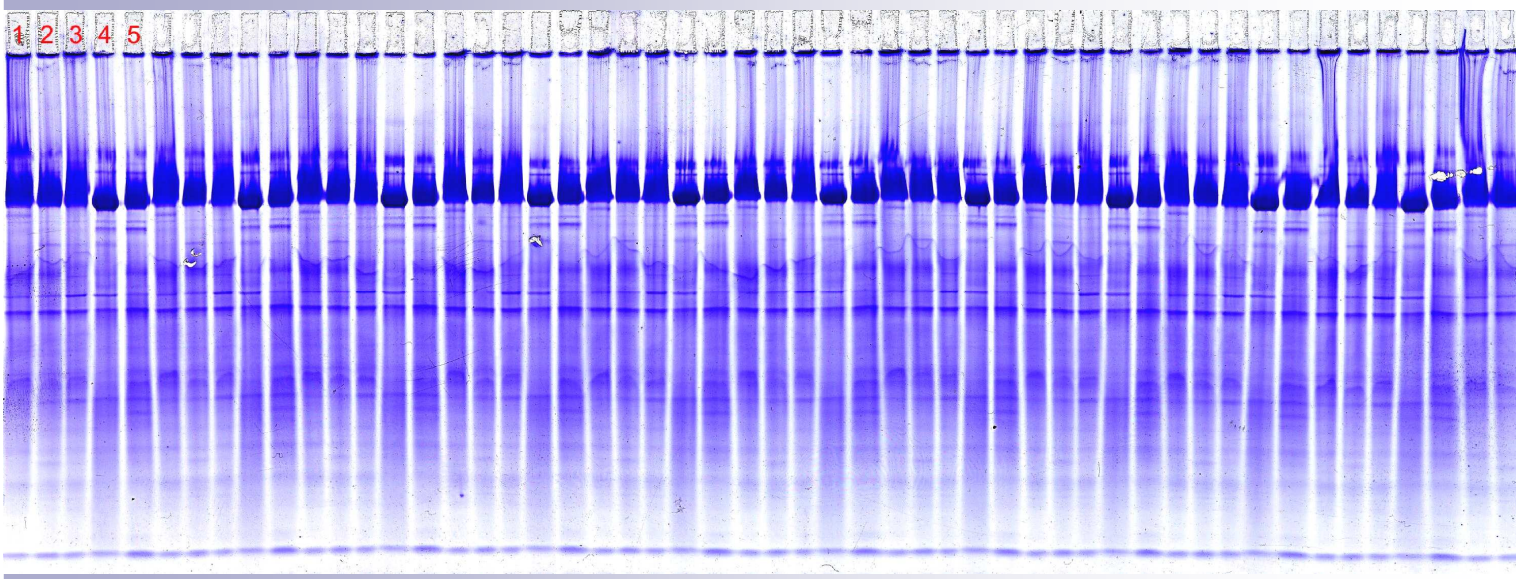

Fig.1: Extracts of 5 different Tomato-varieties running in native elctrophoresis. Coomassie staining.

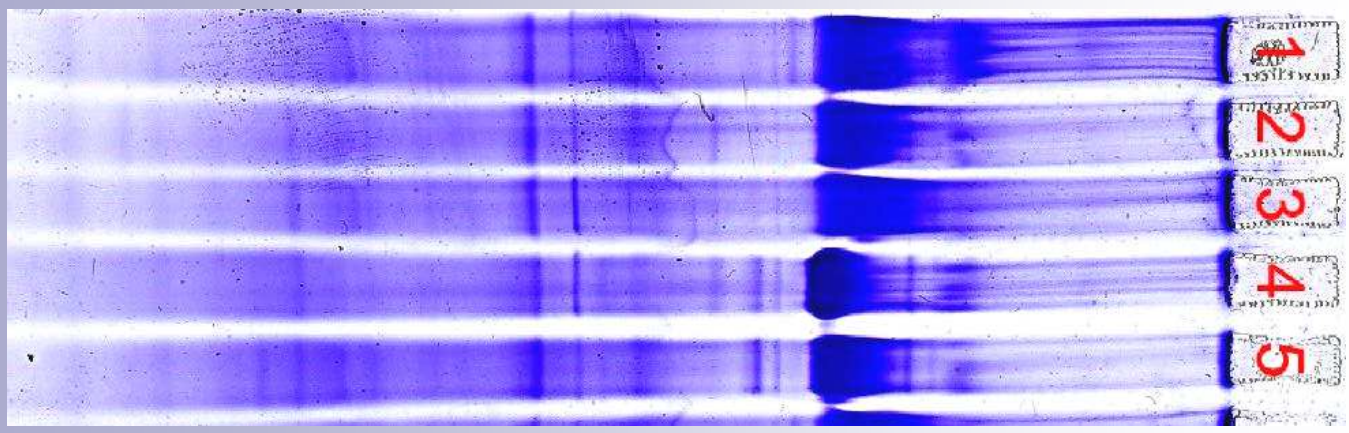

Fig.2: Enlargement of the first 5 lanes.

***General**:* Two major techniques exist for analysis of complex protein mixtures under native conditions, the most common being IEF and Native acrylamide gel electrophoresis.

Isoelectric focusing is based around the formation of a pH gradient typically using carrier ampholytes. IEF however, whilst being a high resolution method does not suit all applications as the carrier ampholytes may have undesirable effect on the proteins to be studied. Some enzymes can be affected and some membrane-protein complexes become unstable during the IEF procedure. Therefore the alternative of native acrylamide gel electrophoresis provides the solution.

In order to achieve the best resolution on native gels an optimized buffer system needs to be applied. The "Protein Buffer Kit Anodic" together with the "DryGel Elpho 12.5% 52S" from EDC due to its neutral pH, produces extremely sharp bands without having a negative impact on the sample proteins.
In the following procedure we describe this buffer system being applied for the analysis of proteins from Tomato leaves. Please note that when following this method be sure to use the detergents specified as well as ensuring the sample protein concentration is within the specified limit.
In general you will find the gel electrophoresis and the staining both quick and convenient.

Hardware

flatbed IEF professional	edc-ief-2836
Staining Tray Normal	edc-wm-n1
DryPool Combi	edc-me-d

Consumables

DryGel Elpho 52S (à 5 µl) 4 gels	edc-411*3*
Protein Buffer Kit Anodic	edc-5003

Rehydration & Electrode Buffers, Electrode Strips, Drying Cardboards, preserving sheets.

Method:

Extraction Buffer

250 mM TRIS	3.04 g
190 mM Glycine	1.5 g
1 mM EDTA	30 mg
0.01 % Dodecylmaltoside [1]	10 mg
0.005 % ProSolv II* (1%)	500 µl

Fill up to 100 ml with H_20 bidist.

Relation Leafs to Extration Buffer Volume

1.5 g leafs + 1 ml extraction buffer

Protein Extraction

1. Pipette 1 ml extraction buffer in a marter
2. Add 1.5 g cutted leafs
3. Work 3 min with the piston
4. Ultrasonic treatment for 10 min
5. Centrifuge 13000 rpm for 5 minutes
6. Transfer 1ml of the liquid phase in a new cup

Sample Dilution Buffer

Gel Rehydration buffer	20 ml
0.001 % ProSolv II *(1%)	20 µl
0.01 % Dodecylmaltoside [1]	2 mg
Orange G (1%)	100 µl
Cochineal Red (1%)	200 µl
Bromophenolred	80 µl

Final volume is about 20.4 ml (res. 21 ml for BN)

Sample Dilution

Light green leafs: 100 + 100 µl, dark green leafs: 100 + 400 µl

Defatting and final Centrifugation

To the samples 20 % (vol/vol) Kerosene is pipetted. Shake well and leave for 10 min. Then pipet the lower hydrophilic phase to a new cap. Finally centrifugate once more 5 min 13000 rpm.

Gel Buffer (rehydration procedure, see next page)

Rehydration buffer	50 ml
Dodecyl-maltoside [1]	10 mg (=0.01%)
Triton X100 (1%)	100 µl (0.002%)

DryGel-Treatment
For the handling of the DryGel´s rehydration in the DryPool Combi (fig.3), see the manual coming with the buffer-system.

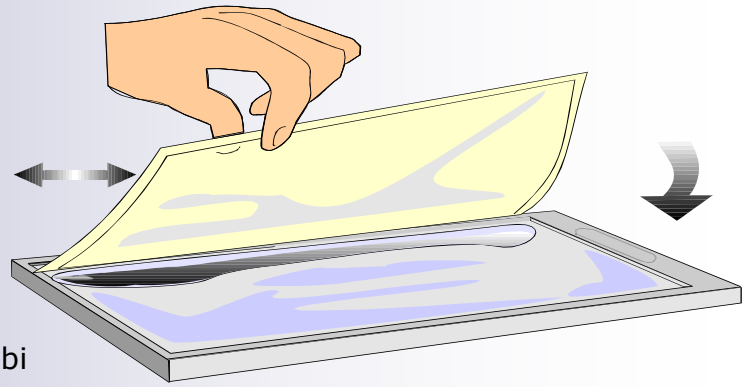

Fig.3: Rehydration of a DryGel in the DryPool Combi

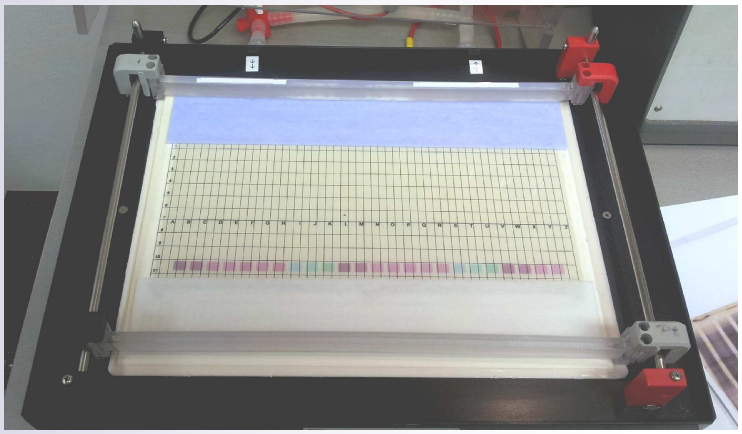

Fig.4: Horizontal chamber: flatbed professional

The Run (15°C)

170 V	10 mA	5 W	25 min
375 V	30 mA	20 W	50 min
450 V	30 m	20 W	40 min

Staining
Hot Coomassie-Staining as general protein staining
General protein staining with Coomassie R-350 in hot acetic acid, see fig 5.
This hot Coomassie-staining is staining and fixing simultanuously!
The acetic acid for staining and destaining can be of technical quality.

Staining solution:
0.02% (w/v) Coomassie R-350 (GE 17-0518-01)
1 tablet (corresponds to 0.4 g dye substance) in 12.5% acetic acid. (Use fresh solutions only!)

Destaining solution:
12.5% acetic acid

Impregnating solution:
5% (v/v) glycerol

Staining programme:
30 min fresh staining solution at 60°C (exhauster) while stirring, see fig 5. Use EDC's staining tray!

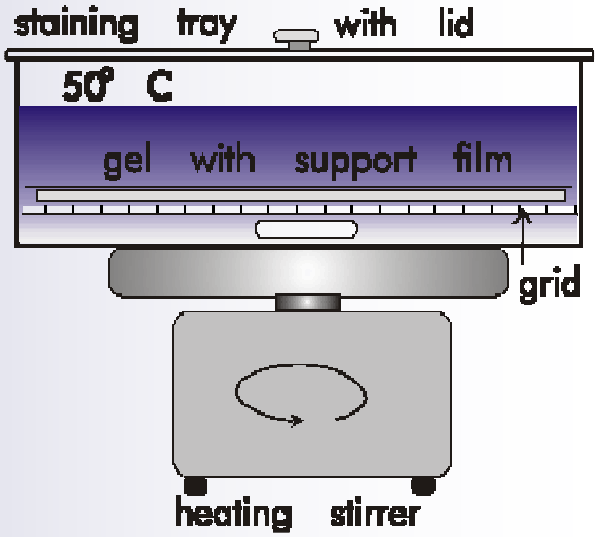

Fig 5: Hot Coomassie-staining

3 x 20 min destaining solution in a tray on a rocking platform.

20 min impregnating solution (tray).
Optimal staining can be achieved when the gel is placed in the first destaining solution overnight at ambient temperature.