Blue-Native Electrophoresis of Plant Leaf-Extracts

09/16

Beside the normally used isoelectric focusing (IEF) native electrophoresis is the other approach to non denaturing protein separation. In which case should IEF be used, when the native electrophoresis? Within an IEF-gel the Ampholytes are needed for building up the pH-gradient , these Ampholytes are not tolerated by every protein and/or they can disturb enzymatic reactions following the electrophoretic procedure. In this cases a native electrophoresis should be performed to avoid these handicap.
Some membrane-protein complexes are not stable during the IEF-procedure.
To achieve a good separation a nice working buffer system should be used:
EDC´s „Protein Buffer Kit Anodic" produces sharp bands, and due to the neutral pH-value the proteins will not suffer during the electrophoretic process. This separation can be used as first dimension for a double dimensional electrophoresis.
The neutral pH-value is also a necessary condition for performing the Blue Native electrophoresis [2], normally used electrophoretic buffers are more basic [1].
If the general protein staining should be quick and easy, then the native electrophoresis gels are more convenient.

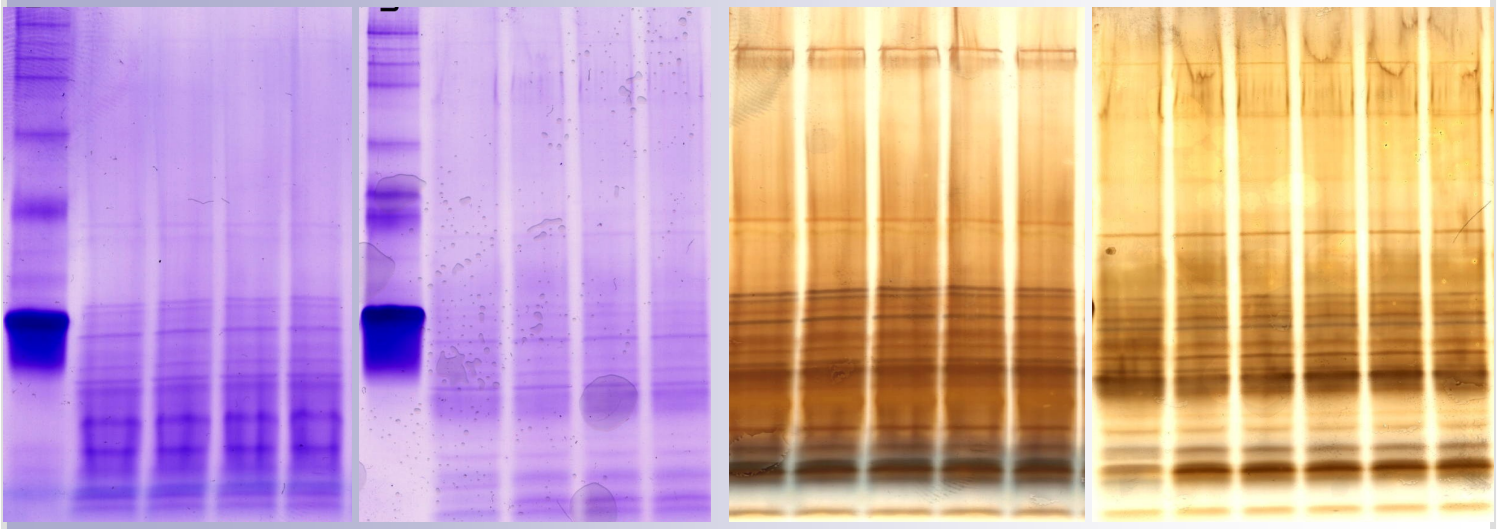

Fig. 1: Serum & Spinach leaf extract in native (left side) electrophoresis and in Blue Native (right side) electrophoresis. Coomassie and silver-staining

Extraction Buffer

250 mM TRIS	3.04 g
190 mM Glycine	1.5 g
1 mM EDTA	30 mg
0.01 % Dodecylmaltoside (DDM)	10 mg
0.005 % Triton X100 (1%)	500 µl

Fill up to 100 ml with H_20 bidist.

Sample Buffer

Rehydration buffer	20 ml
0.001 % ProSolv II *(1%)	20 µl
0.01 % Dodecylmaltoside (DDM)	2 mg
Orange G (1%)	100 µl
Cochineal Red (1%)	200 µl
Bromophenolred	80 µl
Only for BN:	
Coomassie G250 (1%, in Ethanol)	525 µl

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

Consumables Blue Native:

DryGel Elpho 25S (4 gels)	edc-4112
Protein Buffer Kit anodic (for 4 gels)	edc-5003
Native standard „HMW"	VWR 95016-832

Hardware: *(see page 2)*

flatbed professional	edc-prof2836
DryPool combi (for gel rehydration and soaking the electrodes)	edc-me-d
Staining Tray	edc-wm-n1

Protein Extraction

1. Pipette 300 µl Extraction Buffer in a Eppendorf cup
2. Fill the cup with the homogenisated leafs
3. Mix thoroughly with a spatula
4. Ultrasonic treatment for 15 min
5. Centrifuge 13000 rpm for 5 minutes
6. Transfer ~1ml of the liquid phase in a new cup
7. Pipet 200 µl Kerosene on the liquid, shake well and leave for 5 minutes
8. Transfer 0.9 ml of the hydrophilic phase in a new cup
9. Add 100 µl of Sample Buffer

Blue Native Electrophoresis

Add 4 ml of the 1% ethanolic Coomassie G250 solution to 20 ml of the cathode buffer. During the run the gel should look like in figure 2.

References:

[1] Ornstein L.Ann NY Acad Sci. 121 (1964) 321-349
[2] Hermann Schägger: A Practical Guide to Membrane Protein Purification. Academic Press (1994)

DryGel-Treatment
For the handling of the CleanGels, rehydration (fig.3) etc, see the manual coming with the buffer.

R:unning Conditions

			10°C
200 V	20 mA	10 W	20´
375 V	30 mA	20 W	50´
475 V	30 mA	20 W	65´

Total running time is 2 h 15 min

Hardware
Recommended hardware is EDC´s flatbed professional (figure 5) or General Electric´s Multiphor.

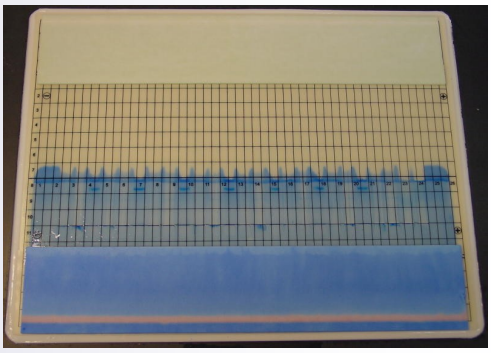

Fig.2: The cathodic side of the gel turns blue during the run.

General Visualization of the Electrophoretic bands
General protein staining with Coomassie R-350 in hot acetic acid, see fig 4.
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 (General Electric 17-0518-01), 1 tablet (corresponds to 0.4 g dye substance) in 10% acetic acid. (Use fresh solutions only!)
Destaining solution:
10% acetic acid
Impregnating solution:
10% (v/v) glycerol
Staining programme:
30 min fresh staining solution at 60°C (fume hood) while stirring, see fig 4.
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 staining solution/10% HAc overnight at ambient temperature.

For silver-staining recipes please visit EDC´s homepage:

www.electrophoresis-development-consulting.de

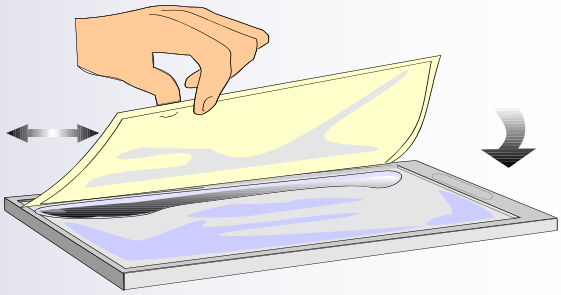

Fig.3: Rehydration of a DryGel in a DryPool combi

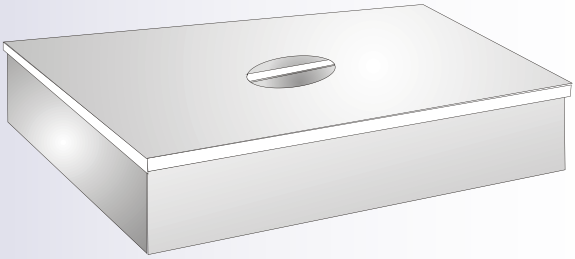

***Fig.6*:** Staining Tray, standard size

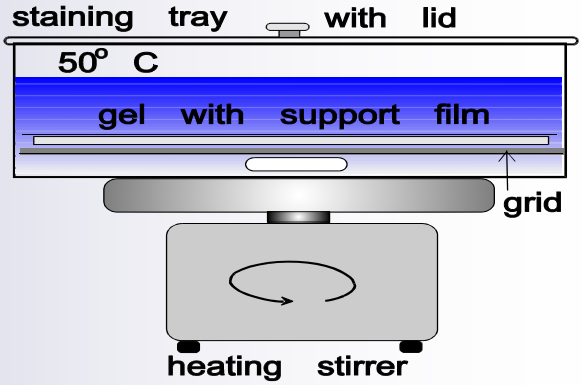

Fig 4*:* Hot Coomassie-staining

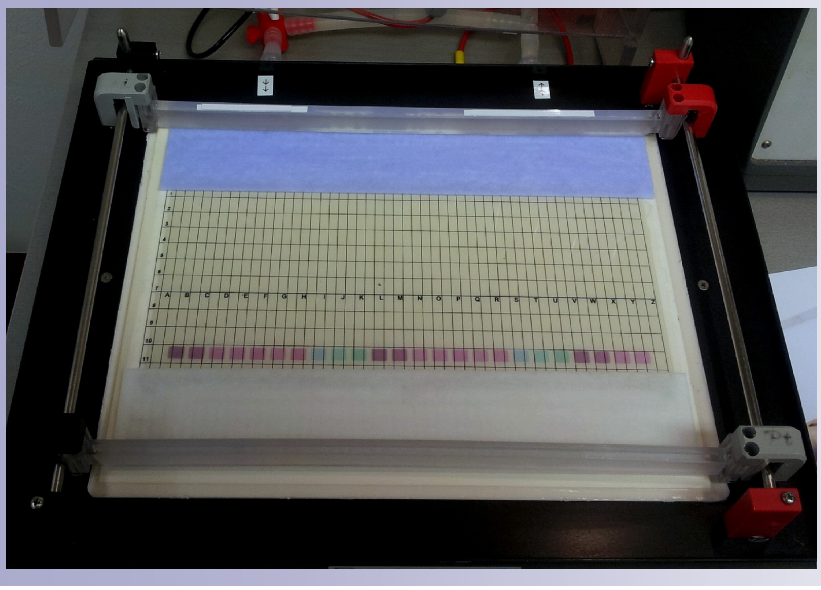

***Fig.5*:** flatbed professional horizontal chamber