# Cereal Cultivar Differentiation with the Sortype Kit

EDC V9 02/18

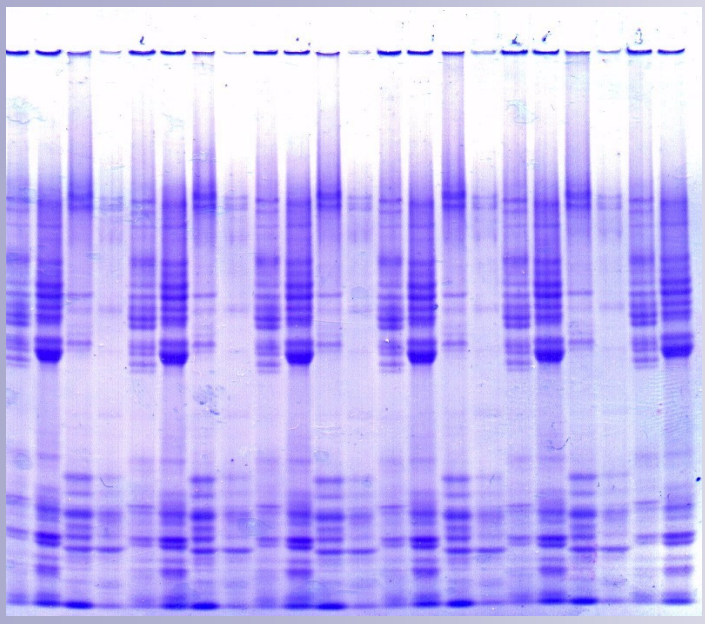


***Fig.1a:*** Sortype Kit: Prolamins of different wheat and barley species after the fast extraction. hot Coomassie R 350 staining

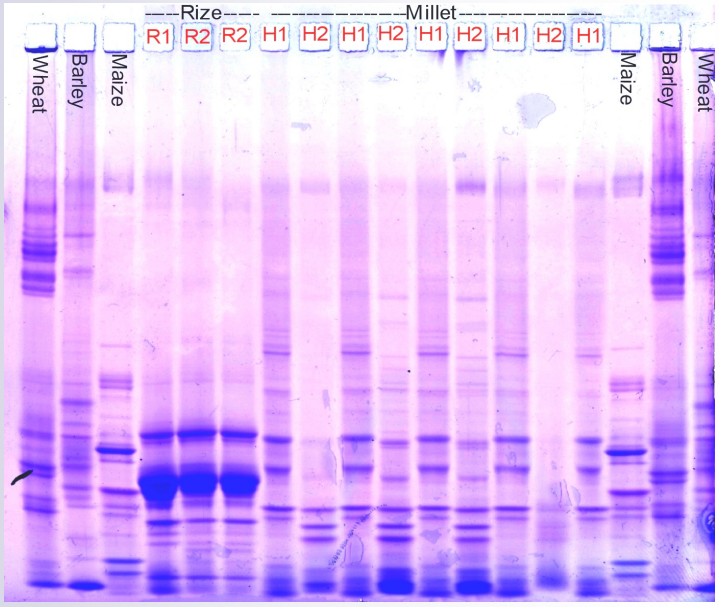



***Fig.1b:*** Sortype Kit, Prolamins of different mellit, rice and maize varieties (hot Coomassie stain)

***General:***
This kit is used for the acidic electrophoresis of positively charged substances in cathodal direction with the DryGel system. This acidic electrophoresis is mostly applied for separations of lipophilic proteins such as alcohol- or chloroethanol- soluble extracts from barley, wheat, and their malts for variety differentiation [1], fig. 1a (Prolamins).
Also suitable for the separation of mellit, rice amd maize varieties see picture 1b.
The solubility of these proteins can be improved by adding urea and nonionic detergents to the rehydration solution. 2 protein extraction methods are suggested: An 55% isopropanolic extraction with several steps for malt and a more simple, rapid extractions for barley, wheat, mellit, rice and maize which employes 30 % Chloroethanol. With hot Coomassie R 350 staining the alcohol soluble prolamins are fixed so efficiently in the matrix, that no trichloroacetic acid is needed.

Sortype Cereal Cultivar Differentiation Kit for Cathodal Electrophoresis consists of:
**Rehydration buffer**,**Cathode buffer**, **Anode buffer, Sample buffer** :
**4 DryGels** for 52 samples of 5 µl volume, 0.5 mm
**4 Drying Cardboards**
**Electrode strips:** 8 electrode strips 5.0 x 25.3 cm.

Additionally needed:
**DryPool Combi** (edc-me-d)
Tray for rehydration of dry gels (normal size)
and soaking electrode strips

**Steel Tray Small** (edc-wm-n1) for hot Coomassie staining
**Urea** (Merck 8488)

**Protein Extraction**
**Fast method** (quick - does not work for malt!):
Pipet the given volume bidest over one crashed grain. 15 min sonification at 40°C. Centrifuge for 5 min with 13 000 rpm, remove water out of the cup. 15 min sonification at 40 °C with 30% Chloroethanol/70% Sample-Buffer-mix (CE,+/- 0.1% DTT). Centrifuge for 5 min with 13 000 rpm

| Step | Wheat | Barley | Maize | Rice | Millet |
|---|---|---|---|---|---|
| **Water** | 500 µl | 500 µl | 300 µl | 150 µl | 60 µl |
| **Extraction** cracked grain | 250 µl (-DTT) | 200 µl (+DTT) | 150 µl (-DTT) | 80 µl (-DTT) | 30 µl (+DTT) |
| **Extraction** grated grain | 800 µl (-DTT) | 600 µl (+DTT) | 500 µl (-DTT) | 250 µl (-DTT) | 100 µl (+DTT) |

**Malt method** (complicated - but the only method for malts)
1.) Pipet to 1 crushed grain: 500 µl (55 % (v/v) isopropanol / $H_2O$dist) and shake well, (+/- 0.1 % DTT see above).2.) 15 min in ultrasonic tray at 40°C. 3.) Vortex the samples for 15 sec, centrifuge for 5 min 13,000 rpm. 4.) Carefully pipet solutions into new tubes. 5.) Remove alcohol for 2 - 3 hours in a SpeedVac under warming the rotor (solutions become turbid).
***Caution!*** Remove isopropanol only: remaining aqueous volume is about 100 µl.
6.) Pipet out and discard the entire supernatant. 7.) Add 0.6 g urea to 10 ml sample buffer (1 mol/l urea) + 10 % Chloroethanol (1 ml / 10 ml, sol.becomes turbid) and add to the pellets. Barley-malt: 140µl (+0.1 % DTT), Wheat-malt: 170 µl. 8.) Vortex the samples for 15 sec to dissolve the pellet to an emulsion. 9.) 15 min ultrasonic tray at 40° C. 10.) Vortex 15 sec and shortly centrifuge 1 min 16000 upm.

**Rehydration of the DryGels**
Rehydration solution: 3 g urea made up to 50 ml with rehydration buffer.
Lay the DryPool Combi onto a horizontal table, fill the 50 ml solution into the chamber. Lay the edge of the gel-support - with the gel surface facing down - into the rehydration buffer (fig. 2) and slowly lower it, avoiding air bubbles. Using foreceps, lift the gel up to its middle, and lower it again without catching air bubbles, in order to achieve an even distribution of the liquid (fig. 3).
2 hours later the gel has reswollen completely and can be removed from the DryPool. Wipe away any urea crystals from the rear surface. Remove buffer completely from the gel surface and the sample wells using on of the drying cardboards.

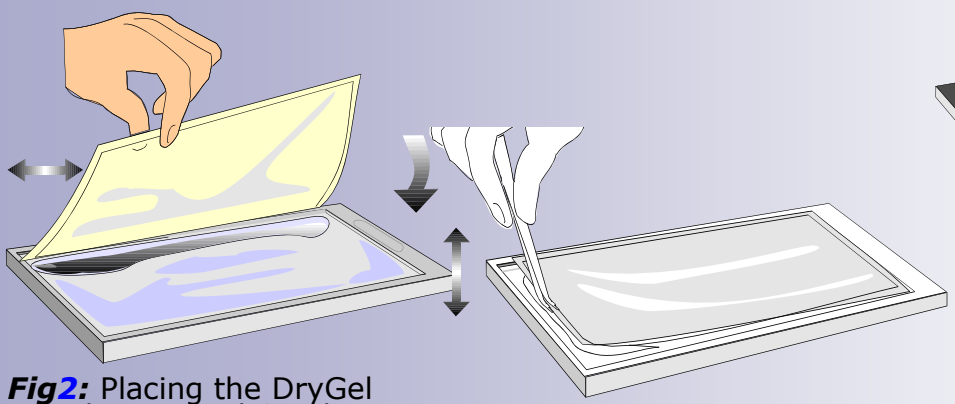
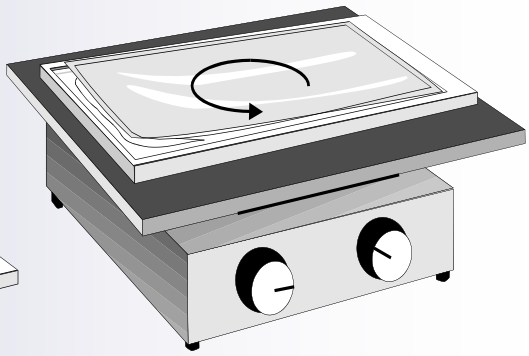


***Fig2:*** Placing the DryGel into the DryPool Combi



***Fig 3:*** Lifting the gel for an even distribution of the liquid…..



....then use a rocking platform

## Application of the Gel and the Electrode Strips

Switch on the thermostatic circulator, adjusted to 20 °C. Spread 1.5 ml of kerosene onto the cooling plate of the electrophoresis chamber, in order to ensure good cooling contact.
Place the gel (surface up) onto the center of the cooling plate: The side containing the sample wells is orientated towards the anode (fig. 6; Multiphor II: cathodal side of the wells matching with line no. 12).
Lay two of the electrode wicks into the 2 long compartements of the DryPool Combi.
Apply 25 ml of the anode and cathode buffers respectively to the strips (fig. 4).
The cathode buffer contains a blue dye to avoid mistakes (this does not spread evenly in the wick).

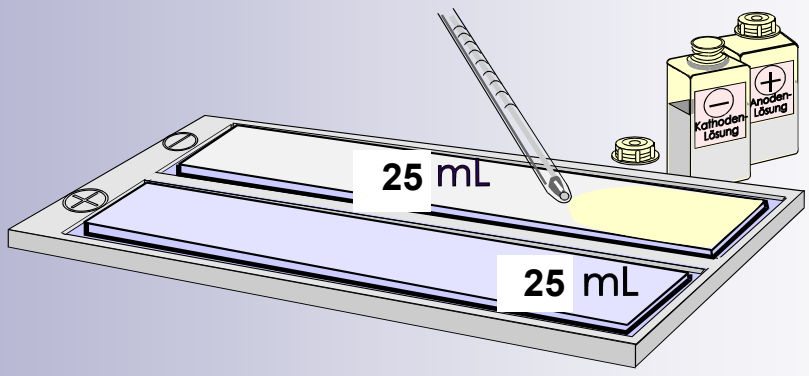



***Fig. 4:*** Soaking the electrode strips with the anode and cathode buffer in the DryPool Combi.

Place the anode strip onto the anodal edge of the gel, matching the grid on the cooling plate (Flattop & Multiphor: between the lines 13 and 14).
Place the cathode strip onto the cathodal edge, matching the grid (Flattop & Multiphor: on line 4). See fig. 5. Always apply anode wick first (more sensitive than the cathode!).
Smooth out air bubbles by sliding bent tip foreceps along the edges of the wicks laying in contact with the gel (first anode, then cathode!).

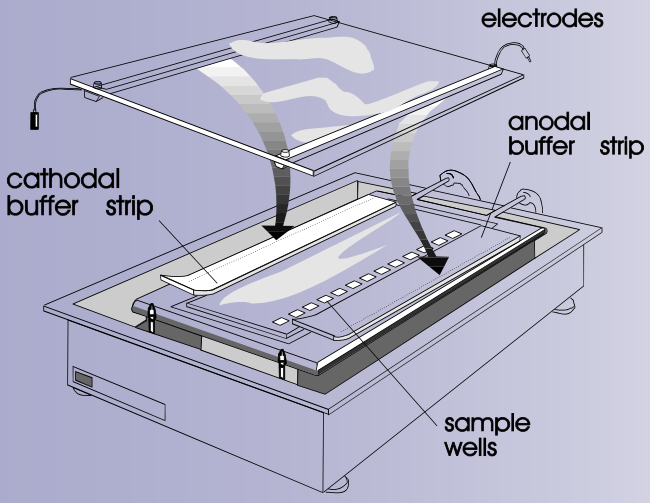



***Fig 5:*** Horizontal electrophoresis apparatus: GE's „Multiphor"

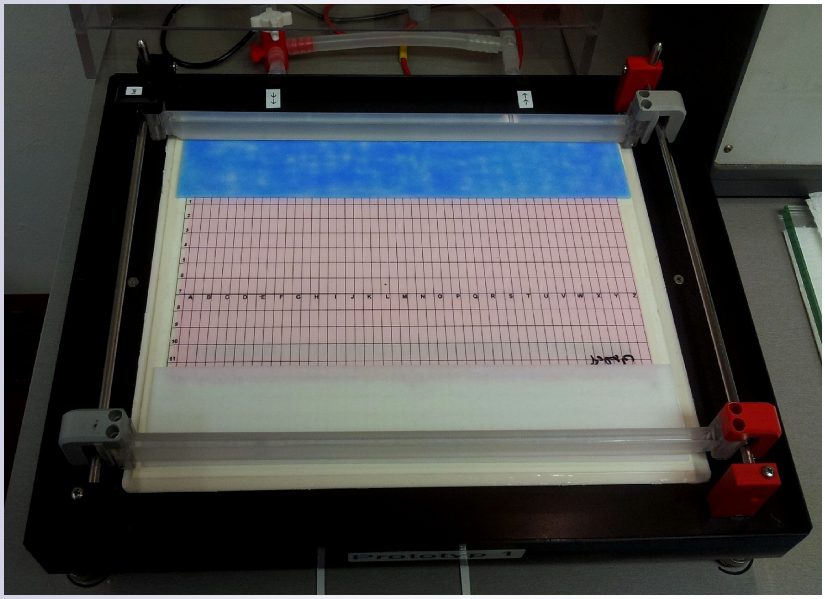


***Fig 6:*** Arrangement of gel, buffer strips and electrodes. Behind the samples is the anode!

**Sample application and electrophoresis**
Apply 5 µl of the emulsion from each sample tube to each well using a micropipette (or use appropriate multipipette, microtiter plate standard distances).
Clean platinum electrode wires before (and after) each electrophoresis run with a wet tissue paper.
Multiphor and FlatTop: Move electrodes so that they will rest on the outer edge of the electrode wicks. Connect the cables of the electrodes to the apparatus and lower the electrode holder plate (fig 6). Close the safety lid.
flatbed professional: Clamp the elctrodes on their steel bar so that they will rest on the outer edge of the electrode wicks.
Pipet the samples. Then set the Heavy Weight Glass-plate on the electrodes, close the lid and start the electrophoresis. See figure 7.

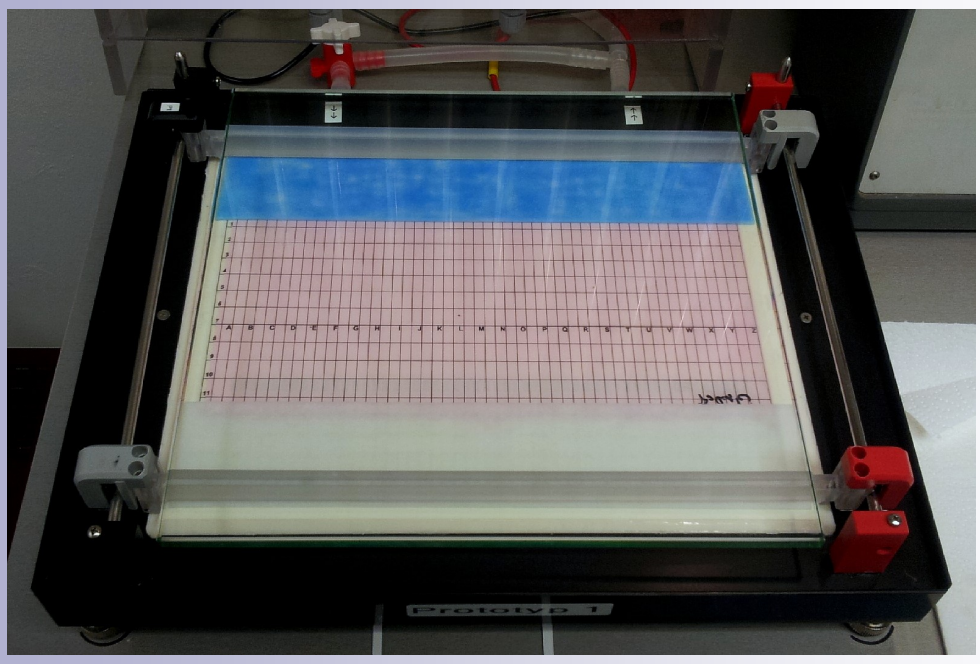


***Fig 7:*** flatbed professional: Arrangement of gel, buffer strips and electrodes. The heavy glasplates ensures good contact.

**Running conditions** (15 °C) max settings of a whole gel

| **Step** | **Volt** | (V read) | **mA** | mA read | **W** | **min** |
|---|---|---|---|---|---|---|
| **1** | 200 | (200) | 15 | (11) | 5 | 30 |
| **2** | 700 | (630) | 24 | (24) | 22 | 30 |
| **3** | 700 | (670) | 35 | (35) | 22 | 45 |

*Half of the gel: Keep voltage and halve mA and W*
The run is complete, when the red buffer front (pyronin dye marker) has reached the cathodal strip.

**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 (Pharmacia LKB 17-0518-01)
2 tablets ( = 0.8 g dye substance) in 2.5 l of 12.5% acetic acid
Use fresh solutions only!
*destaining solution:* 12.5% acetic acid
*impregnating solution:* 10% (v/v) glycerol

Staining programme: 30 min *fresh* staining solution at 50°C (exhauster!) while stirring (fig. 8).
3 x 30 min destaining solution in a tray on a rocking platform, fig 9.
(*Tip: final staining and destaining overnight with:*
*0.003% Coomassie R-350, 0.002% Ponceau S, 10 % HAc)*
30 min impregnating solution (tray).
Air dry overnight

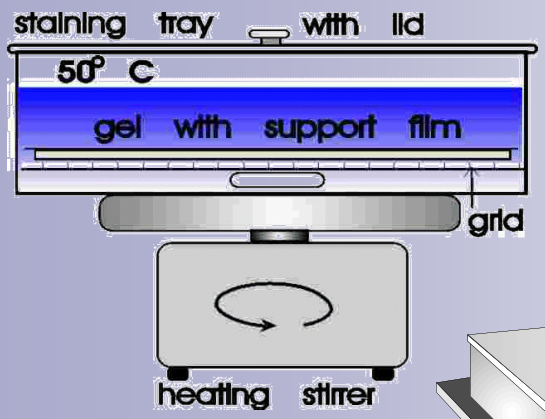



***Fig.8:*** Hot Coomassie-staining

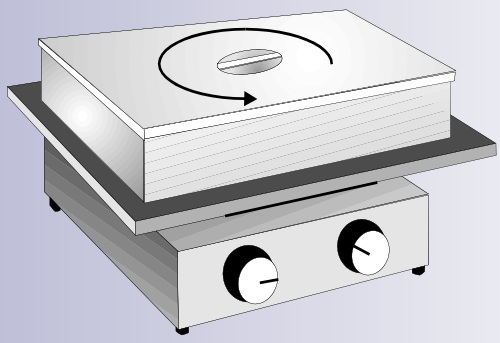


***Fig.9:*** Destaining in a tray

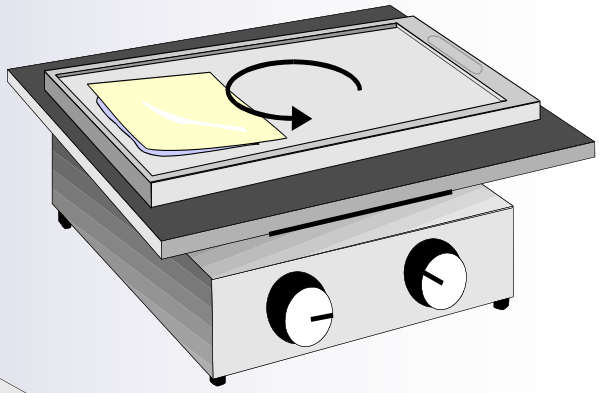


***Fig.10:*** Single step staining

**Quick Staining with Azur Gel Super [2]**: Wash the gel for 5 minutes in dist.water to remove tensides and buffers., figure 10. 2.) Stain the gel in the AzurGel Super solution for 30 minutes. 3.) Before air-drying overnight incubate the destained gel in 10% Glycerol for 30 minutes

**Reference:**
[1] Hsam, S.L.K., Schickle ,H.P., Westermeier, R. und Zeller,F.J.: "Identification of cultivars of crop species by polyacrylamide electrophoresis. I. Commercial barley (*Hordeum vulgare L.*) cultivars grown in Germany." Monatsschrift für Brauwissenschaft *3* (1993) 86-94.

The sample preparation as video clips:
http://www.electrophoresis-development-consulting.de/html/cerealtyping.html
The Sortype Standard Gel with all relevant german Barley varieties of the year 2002:
http://www.electrophoresis-development-consulting.de/html/cerealstandardgelbarley.html
[2] Anamed Elektrophorese GmbH, Ottoweg 10, D-64291 Darmstadt, Tel.: +49/6151/95177-45