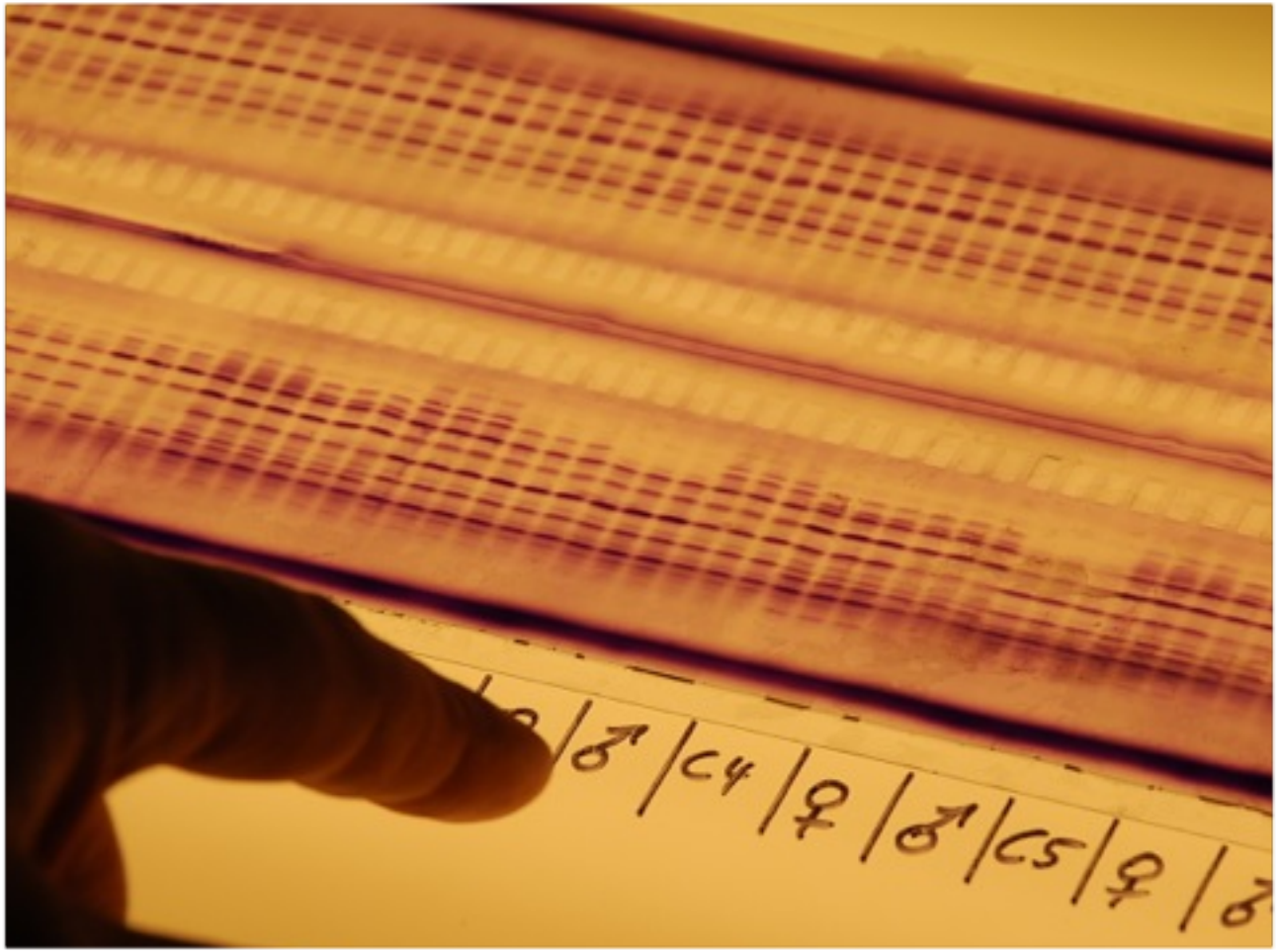
ProTec Bioseparation



HPTK

a new Hybrid Purity Test Kit for Maize

HPTK: a complete routine system for high-throughput genetic purity testing of Maize

A new, fast and powerful method for determining the hybrid purity of maize hybrid varieties

The HPTK system is an electrophoretic procedure tailored to the testing of maize seeds for genetic purity. In maize there are 6 malate dehydrogenase genes that each have several different alleles. The multi-genic and multi-allelic character of MDH makes it possible to distinguish over **one thousand** different combinations of MDH isoenzymes. This allows testing of almost every hybrid variety for inbreds. Furthermore, the high level of variability present in the MDH system enables identification of maize varieties in the majority of cases by evaluating the genotype for the different MDH genes. Thus, in addition to testing for hybrid purity one can also test for undesired pollination or undesired mixing of parent lines.

Besides reliability, the crucial advantage of this test (using the seed or a part thereof) is the speed of its results, since several processes related to seed production, seed cleaning & treatment, packaging and sales hinge strongly on the meeting of deadlines: the results for maize are available within 2 hours.

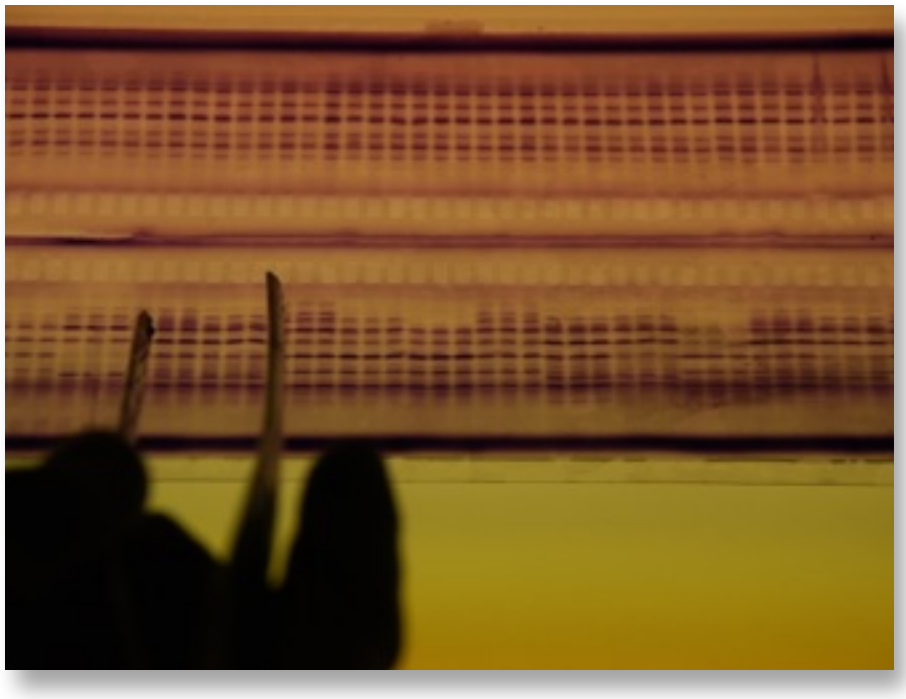
In addition, we offer you a comprehensive solution package, from personal advice on the ideal equipment set-up (sample preparation, electrophoresis chamber, etc.) down to a fully customised detailed protocol.

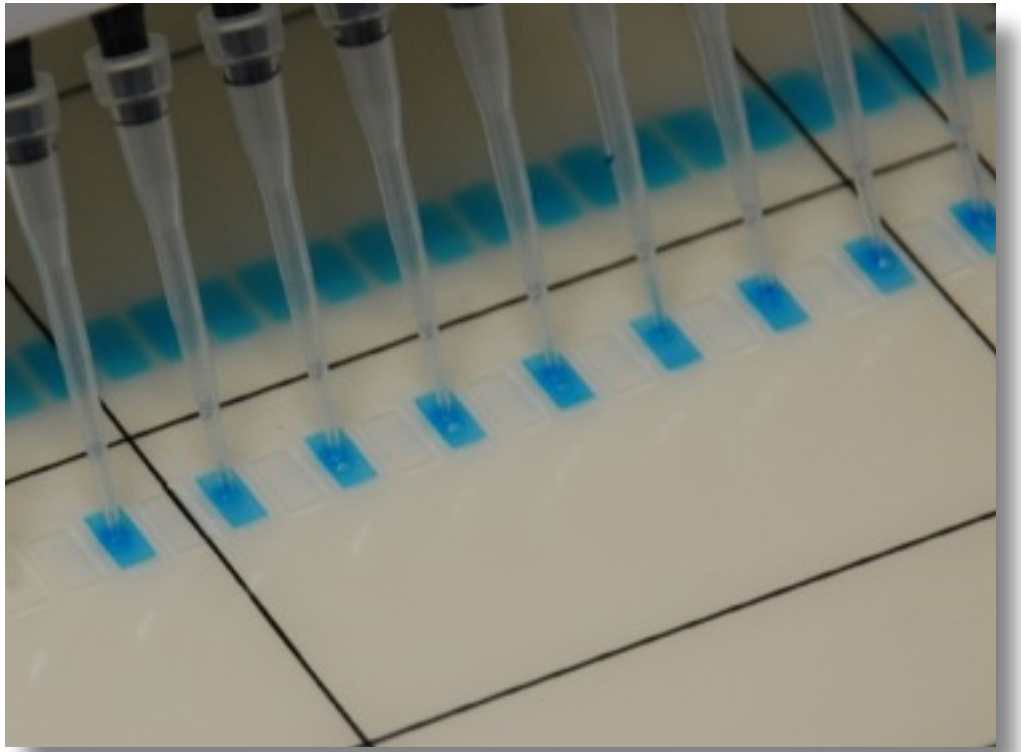
ProTec shows you a simple way to implement an effective quality assurance system.

The principle of isoelectric focussing (IEF)

Electrophoretic methods are based on the migration and separation within an electric field of electrically charged particles such as proteins/enzymes. The particles migrate at different speeds depending on electric charge and size, thus producing a differentiated picture based on the respective migration paths. With isoelectric focussing (IEF) the proteins/enzymes become separated in a cumulative pH gradient. The procedure requires so-called ‚carrier ampholytes' (ampholytes) to build up this gradient. The varying electric charges of the proteins cause them to migrate to the pH value that corresponds to their isoelectric point (pI). Here they ‚lose' their outer charge and become concentrated (focussed) in the form of a narrow band. The great advantage of isolectric focussing is the sharpness of the bands obtained.

Such sharp definition even allows separation of those proteins/ enzymes whose isoelectric points (external charge of the protein) differ by less than 0.01 pH units.





Description :

The ***ProTec Kit*** consists of a ready-polymerised, dried poly -acrylamide gel, equilibrated in an especially provided amph-olyte solution. Into this gel, two rows have been polymerised, each comprising 52 slots. This enables 104 maize samples to be tested in a single run. Also supplied with the kit are ready-to-use MDH enzyme staining tablets along with appropriately optimised MDH extraction buffer.

ProTec Bioseparation

Overview of procedure : 1 - 18

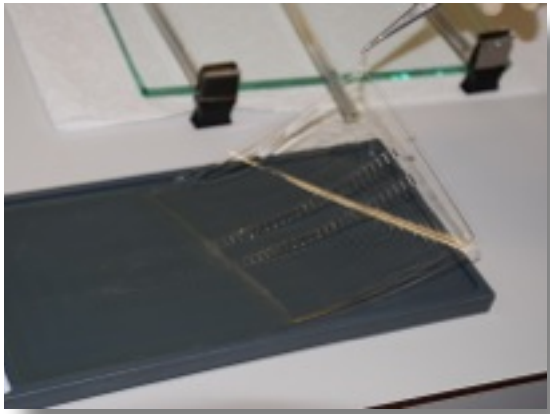
1

Equilibrate the dry gel overnight in 25 ml amph-olyte solution (gel side up), taking care not to trap air bubbles.

2

Remove the moist gel from the tray and dry the tray carefully.

3

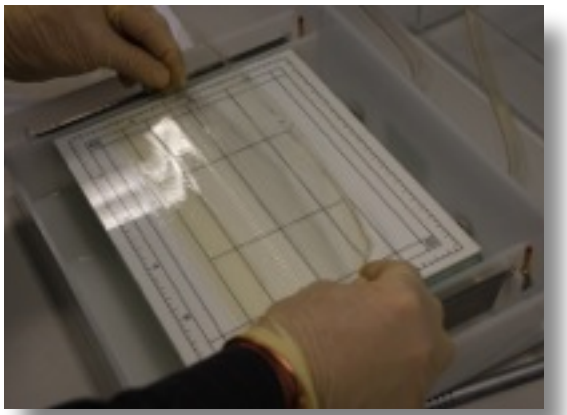


Transfer the moist gel back to the dried tray (gel side down!) .

4

Spray plenty of silicone onto the cooling plate of the electrophoresis chamber .

5



Lay the IEF gel on the cooling plate (gel side up), taking care not to trap air bubbles.

6

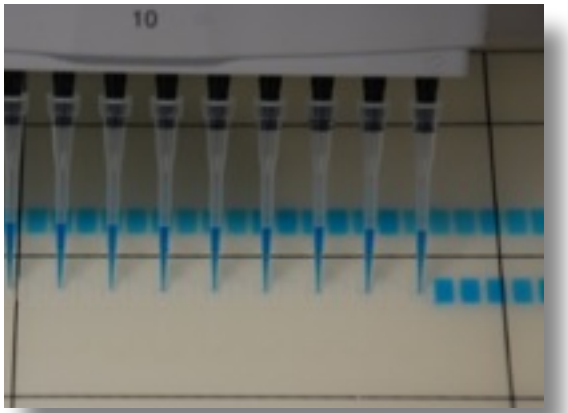
Using a 96-well microplate, vigorously agitate maize samples in extraction buffer (0.7 ml per well) for 1 h . Then leave to settle for 1 h.

ProTec Bioseparation

7

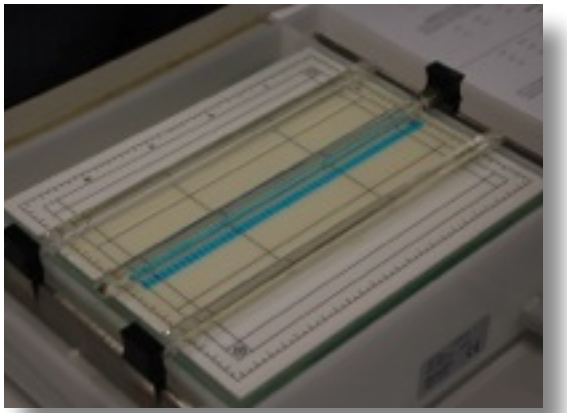
Take 6 µl of extracted maize sample per well, free from foreign particles, from a 96-well microplate using a 12-channel multipipette and ....

8



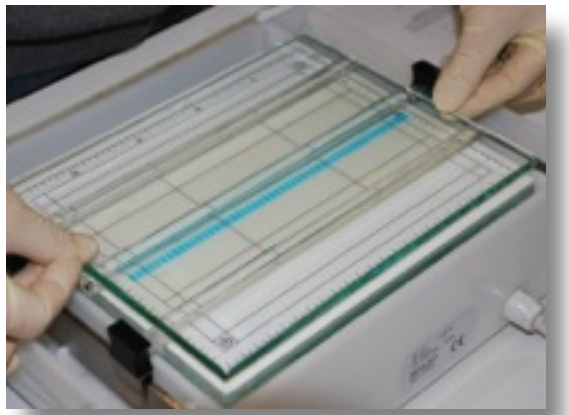
... apply to the 104 slots in the IEF gel.

9



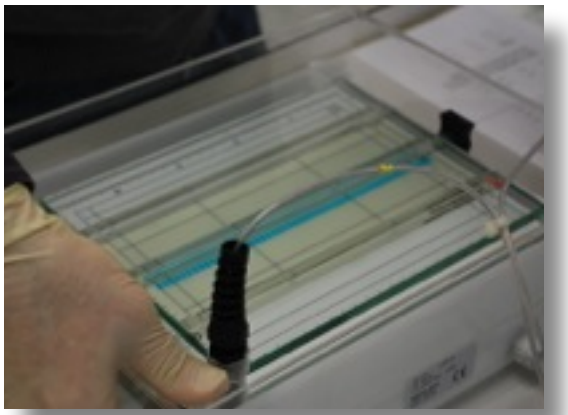
Place 3 electrodes onto the gel:
1 electrode in the middle = cathode;
2 electrodes at the far ends = anode

10



Weight the electrodes with a glass plate (8 - 10 mm) and press down carefully. Good contact to the gel is essential.

11



Close the electrophoresis chamber.

12

Start the electrophoresis program me

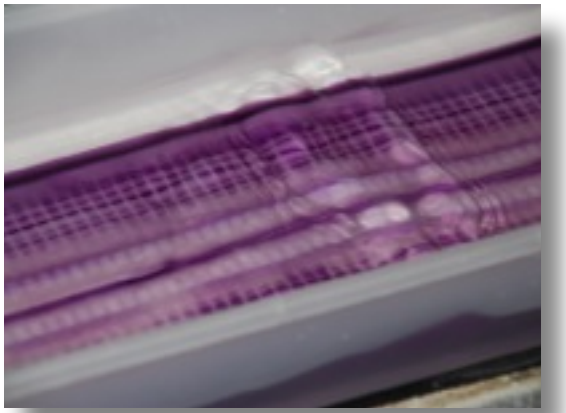
13

MDH staining is complete after 20-25 min.

14

Dispose of staining solution in the appropriate way.

15

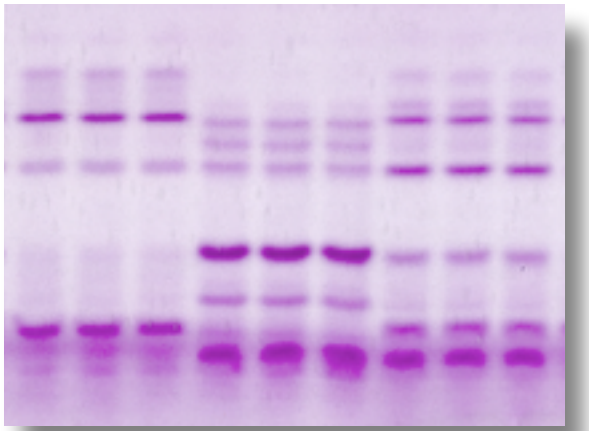


Destain gel as described in the protocol and hang up to dry overnight.

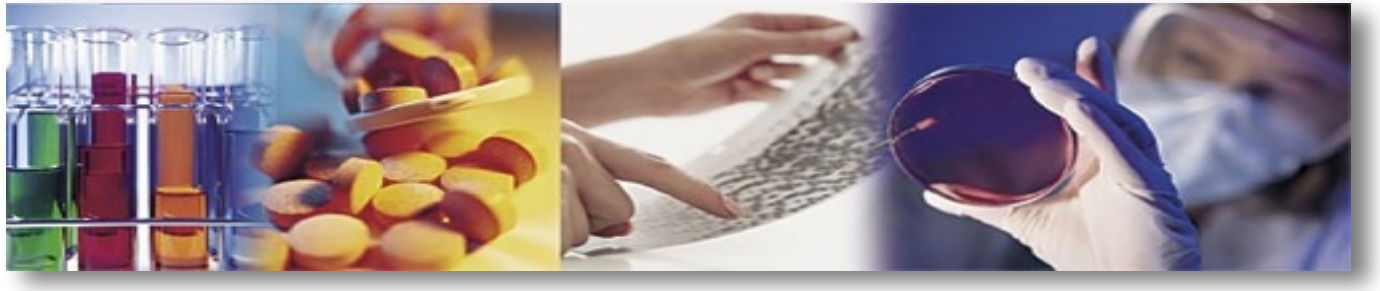
16

The MDH IEF gel is now ready to be analysed.

17



♀ ♂ Mais-Hybrid



Compiled by:

Manfred Demharter
ProTec Bioseparation UG&Co.KG
Wieblinger Weg 100
D-69123 Heidelberg
Germany
Phone: +49(0)6221 7574554
Fax: +49(0)6221 7576057
email: m.demharter@protec-biosep.de