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Test Sample for 2-D Electrophoresis

There is a need for a test sample for 2-D Electrophoresis because of the following reasons:

- Courses
- Demonstration of the Amersham Pharmacia Biotech 2-D electrophoresis equipment and consumables
- Customers want to check their chemicals and 2-D system with a sample, which does always perform.

Such a sample has to

- Be stable at room temperature for almost unlimited time
- Have a high reproducibility of the spot pattern
- Show a heterogeneous multi-spot pattern
- Contain proteins with pIs over a wide pH range (including basic proteins)
- Be easy to use without further equipment (as centrifuge, etc.)
- Usable in Silver Stain and Coomassie Blue concentration

Content of the reaction cup

Carrot seed powder	30 mg
Urea (8 M)	480 mg
CHAPS (4 %)	40 mg
DTT (60 mM)	8 mg

To be stored in the refrigerator (4 to 8 ° C)

Preparation

Before the first use:

- Add 13 µl Pharmalytes 3-10, or IPG buffer according to the pH gradient of the IPG DryStrip used.
- Add 640 µl H_2O_{dist}

Mix thoroughly until the urea has completely dissolved. Shake several times during a period of 10 minutes. Let the cup rest (upright) for ca. 5 minutes. The seed debris will settle down, centrifugation is not needed. Carefully remove the supernatant (ca. 650 µl) and pipet it into another reaction cup.

This sample is stable in the freezer for several months. To avoid repeated freeze-thawing, it is recommended to divide the sample in aliquots before freezing.

Gel rehydration solution

8 M urea	2.4 g
0.5 % CHAPS	25 mg
0.28 % DTT	14 mg
0.5 % IPG buffer	25 µl
0.007 % Bromophenol blue (0.7% BPB (w/v) solution)	25 µl
With H_2O_{dist} fill up to	**5 ml**

Sample Application

A. In-Gel rehydration of sample

for Coomassie Blue Staining:

Add 5 µl of a Bromophenol blue solution (7 % w/v in H_2O_{dist})
Rehydrate the IPG DryStrip in this solution (350 µl for ***18 cm*** strips).

for Silver Staining:

Add **Rehydration solution** for in-gel sample application:
18 cm: 310 µl Rehydration solution + 40 µl sample
13 cm: 215 µl Rehydration solution + 35 µl sample
11 cm: 175 µl Rehydration solution + 30 µl sample
7 cm: 95 µl Rehydration solution + 30 µl sample

B. Cup loading

Apply respective volume of sample at the anodal side of the rehydrated IPG Dry Strip

C. Running Conditions

Temperature: 20 °C; **Current:** 50 µA per strip; **Power:** max. 5 W

Strip length	18 cm	11 cm	18 cm	11 cm
pH Gradient	3-10 L,NL	3-10 L,NL	4-7	4-7
Rehydration*	10 h	10 h	10 h	10 h
150 V	30 min	30 min	30 min	30 min
300 V	60 min	60 min	60 min	60 min
600 V	60 min	60 min	60 min	60 min
8000 V ***3500 V*****	2 h 15 min ***5 h***	1 h 15 min ***3 h***	5 h 15 min ***11 h 45 min***	2 h 45 min ***6 h***
Vh	17,000	10,000	41,000	21,000

*) In the IPGphor the rehydration can be performed under voltage: 5h at 30V + 5h at 60 V.
**) Multiphor conditions

Reference:
Posch A, van den Berg BM, Burg HCJ, Görg A. Genetic variability of carrot seed proteins analyzed by one- and two-dimensional electrophoresis with immobilized pH gradients. Electrophoresis. 16 (1995) 1312-1316.