ProteoChrom®
HPTLC Cellulose sheets, 10x10cm
Cat. No. 1.05651.0001

1. Introduction

Merck ProteoChrom® HPTLC Cellulose sheets are especially developed for fast and easy 2-dimensional separation of peptides and protein digests e.g. peptide mapping (2DPM) including 2-dimensional phosphopeptide mapping (2DPPM).

ProteoChrom® Cellulose sheets are based on optimized microcrystalline cellulose providing separation characteristics highly efficient for 2-D separations. Dedicated separation and staining protocols for 2-dimensional peptide maps are included. For 2- dimensional phosphopeptide maps using ^{32}P labelling the separation protocol (8.1 – 8.3) can be used.

2. Package Content

25 ProteoChrom® HPTLC Cellulose sheets, 10 x 10 cm, 100 µm
1 User protocol

3. Storage conditions

Sheets should be stored on a dry place at room temperature
Protect from moisture and lab atmosphere.

4. Samples

Typical sample concentration:	2 µg / µl
Sample volume:	1 - 10 µl

Detection limits:

Staining with ninhydrin:	50 – 100 ng
Staining with fluorescamin:	1 – 10 ng

5. Materials required but not provided

5.1 For sample application:

TLC auto sampler (e.g. Linomat V, CAMAG).
Alternative: Micro-capillaries (e.g. 2 µl).

5.2 For development:

- TLC chamber for 10x10 cm plates
- Deionised water
- 2- Butanol Merck Art. 1.09630
- Pyridine Merck Art. 1.09728
- Acetic acid 100% Merck Art. 1.00063
- Ammonia 25% Merck Art. 1.05432

5.3 For staining:

Typical TLC equipment for spraying and dipping (e.g. Merck TLC sprayer Art. 1.08540, TLC plate heater).

- with ninhydrin:

- Ninhydrin Merck Art. 1.06762
- 2- Propanol Merck Art. 1.01040

- with fluorescamin:

- Fluorescamin Fluka, Art. 47614
- Triethylamine Merck Art. 8.08352
- Acetone Merck Art. 1.00020

5.4 For documentation

Ninhydrin stain: Imaging system for remission at visible light or plane scanner

Fluorescamin stain: Imaging system for fluorescence remission at UV366 nm

6. Preparation instructions and technical hints

- Use highest purity chemicals & reagents.
- Do not touch layers with fingers.
- Use freshly prepared mobile phase every day.
- Do not use mobile phase for more than one development.

7. Sample preparation

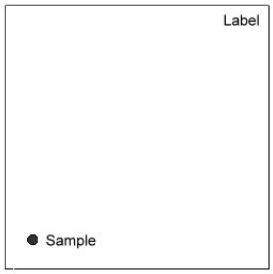
- Samples should not contain caotropic reagents such as Urea.
- When using ProteoExtract® All in One Trypsin Digestion Kit for protein digestion, use extraction buffer 2 instead of extraction buffer 1 protocol B, but with extraction buffer 2).

Protocols

8. Two dimensional separation of peptides and protein digests

Before you start mark one corner of the sheet with a soft pencil.

8.1 Sample application



- with automatic TLC sampler

Use the band wise mode. Set band width 0 – 2 mm and spray with low dosage speed (e.g. 50 nl/s). The application position is 10 mm from the edges in the diagonal corner from the mark. Dry the sheet 5 min at room temperature.

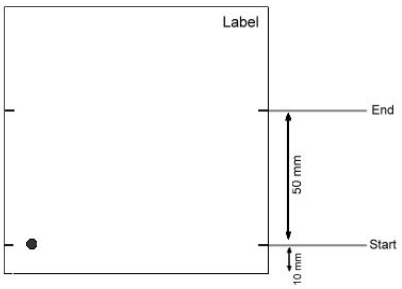
- with capillaries

Fill the capillary with sample. Bring the capillary careful in contact with the layer at the application position 10 mm from the edges in the diagonal corner of the label to apply the sample. Dry the sheet 5 min at room temperature.

8.2 Plate development (separation)

- First dimension

Mark the chromatogram zone on the sheet with a soft pencil at 1- and 6 cm on both edges in the direction of development (see picture).
Prepare the mobile phase and fill the TLC chamber. The level of mobile phase in the chamber should be ~7 mm. Place the sheet in the TLC chamber (without chamber saturation, no filter paper) to start development of the first dimension.
The migration time for 5 cm is approx. 1h. When the mobile phase reaches the end of the chromatogram zone, remove sheet from the chamber and dry it under a nitrogen stream or cold air at least for 30 min.

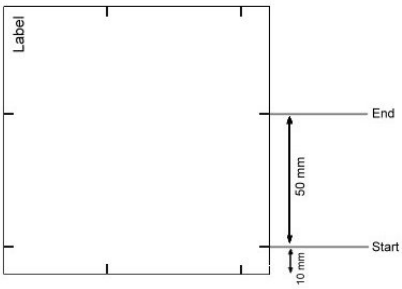


1st dimension conditions
Mobile phase:
2-butanol/pyridine/acetic acid (100%)/ water (30+20+6+24)

Migration distance: 5 cm
Migration time: approx. 1 h

- Second dimension

Turn the sheet 90° and mark the chromatogram zone on the sheet with a soft pencil at 1- and 6 cm on both edges in the direction of development (see picture). Prepare the mobile phase and fill the TLC chamber. The level of mobile phase in the chamber should be ~7 mm.
Place the sheet in the TLC chamber (without chamber saturation) to start development of the second dimension. The migration time for 5 cm is approx. 1h.
When the mobile phase reaches the end of the chromatogram zone, remove sheet from the chamber and dry it under a nitrogen stream or cold air at least 30 min.

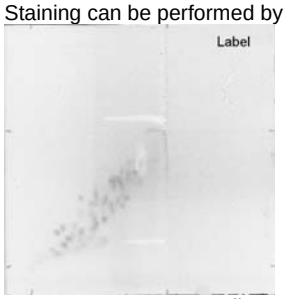


2nd dimension conditions
Mobile Phase:
2-butanol/pyridine/ammonia (25%)/water (39+34+10+26)

Migration distance: 5 cm
Migration time: approx. 1 h

9. Staining of peptides and protein digests

Staining can be performed by spraying or dipping.



Two- dimensional chromatogram
of BSA stained with ninhydrin.

- with ninhydrin

Solution:	0,5% ninhydrin in 2-propanol
Spraying:	Spray the sheet homogenously with ninhydrin solution and heat up to 110°C for 2 min.
Dipping:	Dip the sheet for 1 s in ninhydrin solution and heat the chromatogram up to 110°C for 2 min.
Documentation:	Use an image system at daylight or a plane scanner.

- with fluorescamin

Solution 1:	0,02% fluorescamin in acetone
Solution 2:	10% triethylamine in acetone
Spraying:	Spray the plate homogenously with solution 1 and dry the sheet at RT for 10 minutes. Spray homogenously with solution 2 and dry the sheet at RT for 10 min.
Dipping:	Dip the sheet in solution 1 for 1 s and dry at RT for 10 min. Dip the sheet in solution 2 for 1 s and dry at RT for 10 min.
Documentation:	Use an image system at UV366 nm

10. Related products

ProteoExtract® All in One Trypsin Digestion Kit (Merck Biosciences Art 650212)

11. Trademarks

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