

ScreenTape® P200 provides a simple method for ion exchange chromatography (IEX) fraction selection

ScreenTape P200 automatically resolves and annotates protein peaks found in IEX samples, allowing efficient and straightforward fraction selection. ScreenTape P200 can separate proteins between 10 to 200kDa over a broad range of different concentrations. With this degree of flexibility, the platform lets you study complex protein fractions and allows you to accurately optimise target-protein elution conditions.

Introduction

IEX separates proteins according to charge and is commonly used as a first-pass purification protocol for complex protein samples such as cell lysates and tissue extracts. Consequently, IEX fractions often contain complex mixtures of proteins that can span a wide range of different concentrations. Also, mobile phase composition can vary according to the nature of the sample and the gradient conducted during the elution step, potentially resulting in fractions with diverse salt concentrations or pH values. ScreenTape P200 fully automates the electrophoresis and analysis of IEX fractions. In less than one minute per sample this platform separates 16 complex protein samples and accurately resolves and annotates peaks with values such as molecular weight, peak volume and percent purity.

Tape Used – ScreenTape P200

Time to result for 16 samples

Less than one minute per sample

Sample volume per test – 2µl

Specifications

Sizing range: 10 – 200kDa
Molecular weight accuracy (average deviation) <10%
Molecular weight precision <2% CV
Protein sample concentration: 0.1 to 1mg/ml
Linear range: 2 to 200ng per band
Limit of detection <1ng

Analysis Results

Automated peak location and annotation
Electropherogram overlay for fraction comparison
Automated protein sizing, purity and quantity
GLP standard report and print-out

Materials and Methods

Test samples containing desalted rat muscle extract were fractionated by anion and cation exchange chromatography. Four millilitres of this extract containing 22mg protein were loaded onto a 1ml HiTrap™ heparin column (Cation exchanger - GE Healthcare). The column was washed with eight column volumes (8ml) low salt wash (30mM MOPS pH6.9, 5% v/v glycerol, 0.03% Brij 35, 7mM 2-mercaptoethanol). Proteins were then eluted from the column in 17 one millilitre fractions (named SD3 1 - 17) using a 0 to 1.2M NaCl gradient. Absorbance, conductivity and gradient readings for the purification are shown in Figure 1. The flow through and low salt wash (FT) from the initial loading (combined to be 12ml at 0.4mg/ml), was pH-adjusted to 8.2 with NaOH and applied to a one millilitre Source™ 15Q column (Anion exchanger - GE Healthcare). The column was washed with eight column volumes (8ml) low salt wash (30mM Tris pH8.2, 5% v/v glycerol, 0.03% Brij 35, 7mM 2-mercaptoethanol). Proteins were then eluted from the column in 17 x 0.59ml fractions (named SD4 1 - 17) using a 0 to 1M NaCl gradient. Absorbance, conductivity and gradient measurements for the purification are shown in Figure 1. SD3 samples were diluted 1:5 prior to analysis. A two microlitre aliquot from each of the collected fractions was analysed on ScreenTape P200.

ScreenTape P200 Analysis Procedure

All samples were prepared for ScreenTape P200 according to the Lab901 protocol. Pre-staining of samples during the sample preparation stage avoids lengthy staining and destaining procedures that are common to SDS-PAGE methods. Tubes containing prepared protein samples were placed in the TapeStation® with ScreenTape P200 and tips. After clicking “START” on the software driven menu, full analysis of the samples was achieved and archived, with no user intervention, in less than one minute per sample.

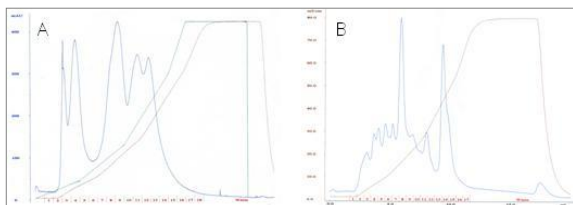


Figure 1: Graphs generated by the ÄktaPrime (GE Healthcare) showing values measured during IEX fractionation of rat muscle extract. Absorbance readings (blue trace), % salt gradient (0 - 1.2M NaCl, green trace) and conductivity measurements (brown trace) are shown for material eluted from the HiTrap heparin cation exchange column (panel A) and Source 15Q anion exchange column (panel B).

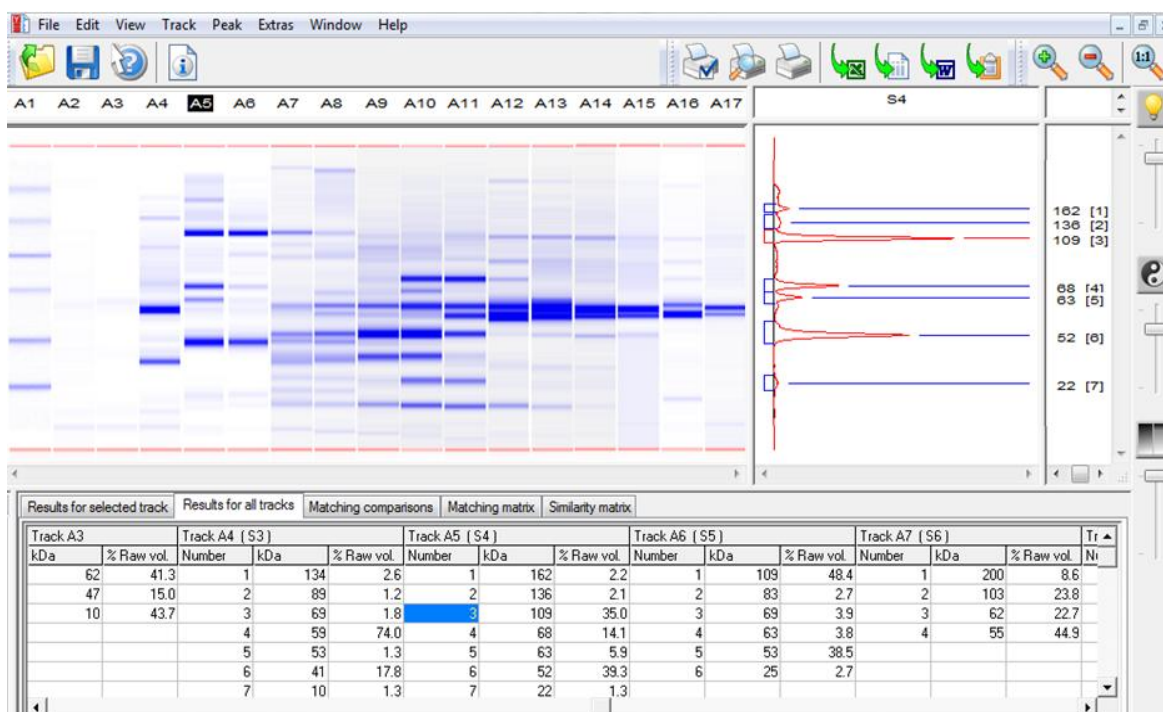


Figure 2: Lab901-GeneTools screen-grab of SD3 fractions 1 to 17. Protein analysis results are automatically presented by the ScreenTape P200 platform, without the need for a manual gel documentation system. All electrophoresis results are presented as a gel image. When a lane is selected it is displayed as an electropherogram (lane 5 has been selected in this view) where protein peaks are annotated with molecular weight (shown here) or quantity. All results are also displayed in a table containing data such as peak number, molecular weight, quantity and % purity.

Results

ScreenTape P200 platform displays fully analysed results for rat muscle IEX samples, which includes a familiar gel image. P200 results also include protein molecular weights, peak purity values and protein quantities, which are automatically calculated and presented in an electropherogram and a table thereby avoiding the use of a manual gel documentation system. This data allows you to compare sample compositions and to easily determine the percent gradient at which your target-protein elutes (Figures 2 and 3). Electropherograms from different samples can also be overlaid for direct peak comparison. The sub nanogram sensitivity and dynamic range of 2 to 200ng/band measured on P200 delivers accurate results for complex IEX fractions. These results are generated without any labour intensive steps and are automatically archived and printed to a GLP compliant report with a single mouse click.

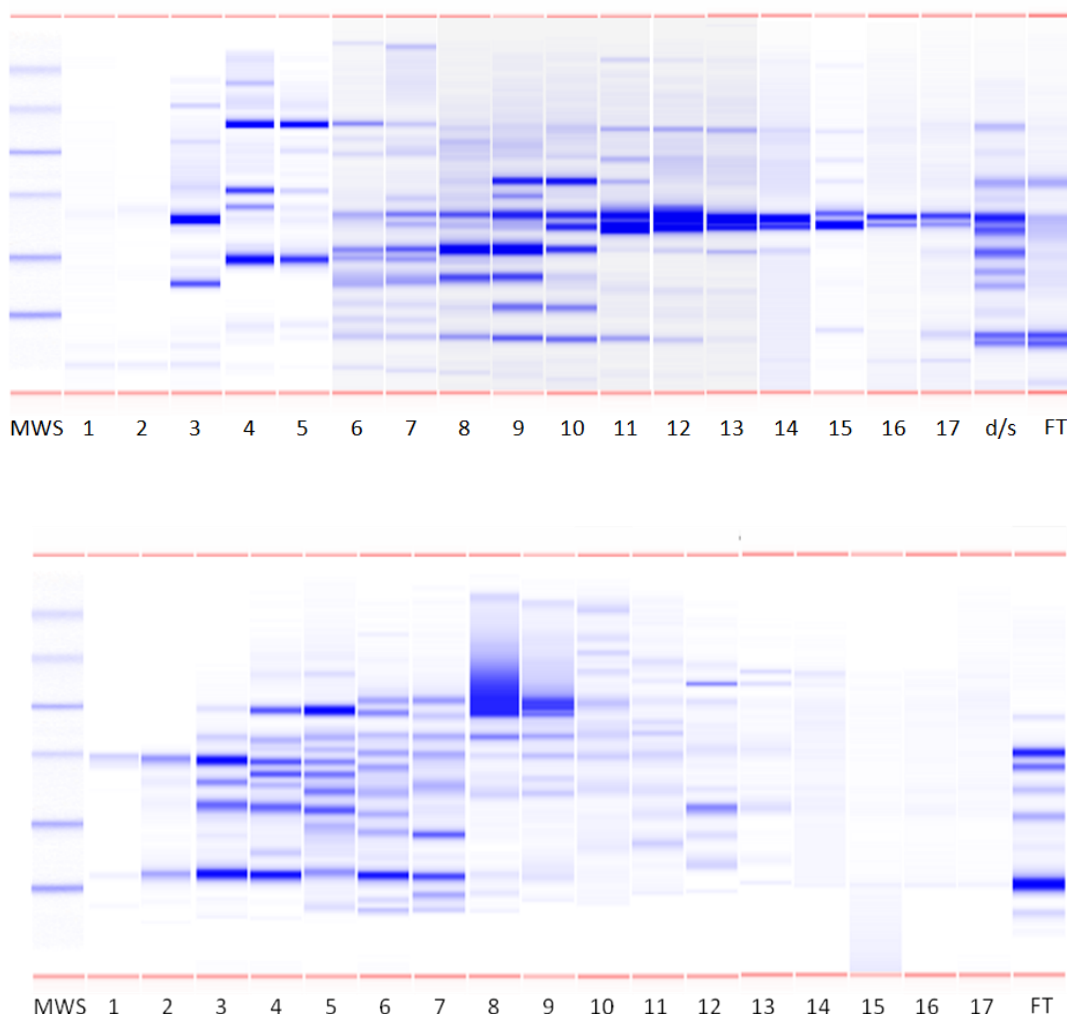


Figure 3: Gel images from SD3 (top panel) and SD4 (bottom panel) fractions 1 to 17 from HiTrap heparin column run on ScreenTape P200. In the top panel FT is the column flow through and d/s the low salt wash, which are combined to form the starting material for the SD4 cation exchange purification. In the bottom panel, FT is the column flow through. MWS denotes molecular weight standards with bands at 200kDa, 120kDa, 85kDa, 60kDa, 50kDa, 30kDa, 20kDa and 10kDa.

Benefits of using ScreenTape P200 for analysing IEX fractions

- **Faster results for IEX fraction analysis** - ScreenTape P200 enables rapid electrophoresis and analysis of IEX fractions. With P200 IEX purification methods are streamlined, allowing you to select relevant fractions more easily and to proceed faster with downstream applications.
- **Take advantage of a more versatile platform** - The sensitivity and dynamic range of P200 allows proteins of very different concentrations to be visualised. This means that low abundance products can be seen alongside high concentration proteins and consequently accurate elution profiles can be determined for target proteins.
- **Improve lab safety** - P200 reagents are pre-packaged making it a convenient and safe electrophoresis method.
- **Increase reproducibility** - Full automation and process control during P200 manufacturing means more reproducible protein sizing than traditional SDS-PAGE giving you better confidence when checking for protein purity.
- **Become GLP compliant** - Electrophoresis results are digitally archived in a GLP compliant format, which enables easy batch to batch comparison and accurate record keeping.
- **Be more economical** - The sensitivity of P200 allows you to use low sample volumes saving precious purified material.

For a full list of application notes covering additional protein purification analysis methods, please visit www.lab901.com.

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