lab901

Application Note

ScreenTape® P200 streamlines the analysis of protein fractions during His-Tag affinity purification methods

ScreenTape P200 is a fully automated electrophoresis analysis method for proteins of 10 to 200kDa. ScreenTape P200 simplifies the screening of protein fractions from His-Tag purifications because it is easy to use and integrates complete sample analysis. With ScreenTape P200 you can analyse 16 protein fractions in less than one minute per sample. This allows you to streamline your protein purification workflow, by shortening the time-to-result on intermediate protein QC checks.

Tape Used - ScreenTape P200

Time to result
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 – 1mg/ml
Linear range: 2 to 200ng per band
Limit of detection <1ng

Analysis Results
Automated peak location and annotation
Automated protein sizing
GLP standard report and print-out

Introduction

Poly-histidines are commonly used to tag recombinant proteins at the N- or C- terminus as a means to achieve rapid product isolation through affinity purification on metal-ion columns. This is possible thanks to the high affinity of histidine for ions such as Cu^{2+}, Ni^{2+} and Zn^{2+}. During the purification process, collected fractions are routinely tested for correct protein content using traditional methods such as SDS-PAGE analysis. ScreenTape demonstrates accurate and reproducible performance for protein fraction analysis and is a method of choice for this procedure. Purified His-tagged proteins are accurately sized, whilst impurities are precisely detected, sized and quantified.

Materials and Methods

The His-Tag purification method used a HIS-Select nickel affinity gel (Sigma) in phosphate buffer (50mM NaH_2PO4, 0.3M NaCl, pH8) with concentrations of 10mM imidazole in the wash buffer and 250mM imidazole in the elution buffer. A His-Tagged protein of 40kDa was prepared at 6.51mg/ml in 25mM Hepes pH7.5, 1mM DTT, 50% glycerol, 1mM benzamidine. Several washes were used to equilibrate the His-Select nickel affinity gel into wash buffer. Three 200µl aliquots of equilibrated gel were re-suspended in either 100µl of diluted His-Tagged protein at 1.3mg/ml in wash buffer (original protein sample), 100µl Bovine Serum Albumin (BSA)/Lysozyme mix at 1mg/ml in wash buffer (the spike), or 100µl BSA/Lysozyme 1mg/ml, His-Tagged protein 1.3mg/ml in wash buffer (contaminated protein sample). These gel-protein suspensions were incubated on ice for 30 minutes with regular mixing. Before the elution phase the gels were washed five times in wash buffer. Proteins were eluted from the nickel resin in 100µl elution buffer. For comparison, protein samples were also prepared for analysis on a 4-12% Bis-Tris NuPAGE® SDS-PAGE (Invitrogen) gel, following the manufacturer's instructions for electrophoresis with MES running buffers.

ScreenTape P200 Analysis Procedure

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

Results

Lab901-GeneTools™ software presents analysed protein results (displayed here in Figure 1) that allow precise monitoring of His-Tag purification products. These results contain information on the protein molecular weights and peak volumes, which are automatically calculated and presented in an electropherogram or a table. Peaks are automatically annotated and can easily be compared between samples (as shown in Figure 3), making it easy to see differences in levels and types of impurities. Each lane contains in-lane markers (red bands seen in Figure 1), which deliver highly reproducible protein sizing. By comparison, the results from the samples run on the NuPAGE system can be seen in Figure 2. These were obtained after 3 hours manipulation with several time-consuming steps, which included buffer preparation, manual gel loading, staining, and destaining. P200 results are available in under one minute per sample and the data is automatically archived and printed to a GLP compliant report with a single mouse click.

Lab901 Ltd, IMEX Business Centre, Loanhead, EH20 9LZ, UK
t +44(0) 131 448 2904 f +44 (0) 131 448 0679 w www.lab901.com

Fractions from nickel matrix purifications can be used directly on ScreenTape P200, even though they contain imidazole concentrations of up to 250mM. This buffer tolerance avoids an extra buffer exchange step prior to sample preparation, which is often a requirement of other microscale automated electrophoresis systems.

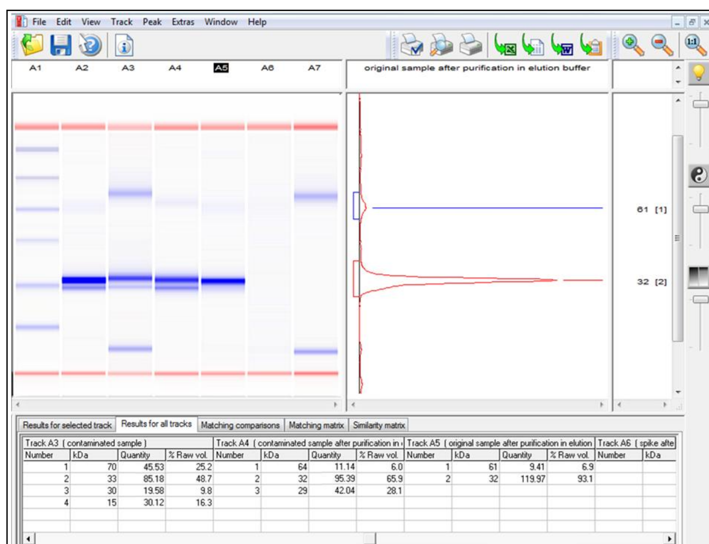

Figure 1: Analysis of HIS-Select Nickel Affinity Gel fractions by the ScreenTape P200 system – Lane 1 contains a broad range molecular standard with proteins at 200kDa, 120kDa, 85kDa, 60kDa, 50kDa, 30kDa, 20kDa, and 10kDa. Lane 2 contains the original protein sample. Lane 3 contains the contaminated protein sample. Lane 4 shows the contaminated sample after purification in elution buffer (250mM imidazole), showing a major band sized at 32kDa. Lane 5 contains the original protein sample after purification in elution buffer. Lane 6 contains the spike after purification in elution buffer. Lane 7 contains the spike.

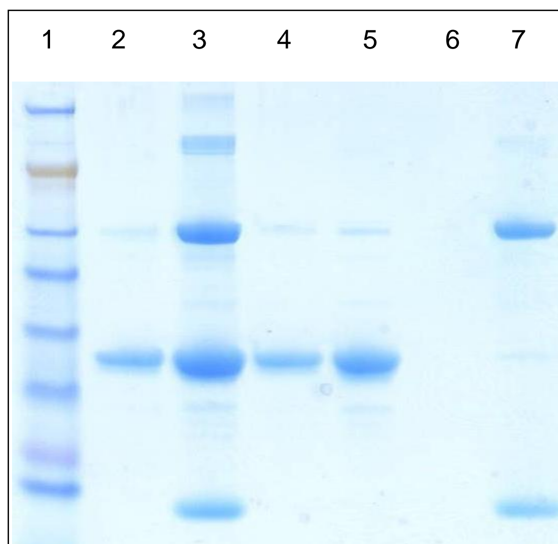

Figure 2: Analysis of HIS-Select nickel affinity gel fractions on a Coomassie stained NuPAGE gel – Lane 1 contains SeeBlue+2 markers with proteins of 188kDa, 98kDa, 62kDa, 49kDa, 38kDa, 28kDa, 17kDa and 14kDa. Lane 2 contains the original protein sample. Lane 3 contains the contaminated protein sample. Lane 4 shows the contaminated sample after purification in elution buffer (250mM imidazole), showing a major band at approximately 32kDa. Lane 5 contains the original protein sample after purification in elution buffer. Lane 6 contains the spike after purification in elution buffer. Lane 7 contains the spike.

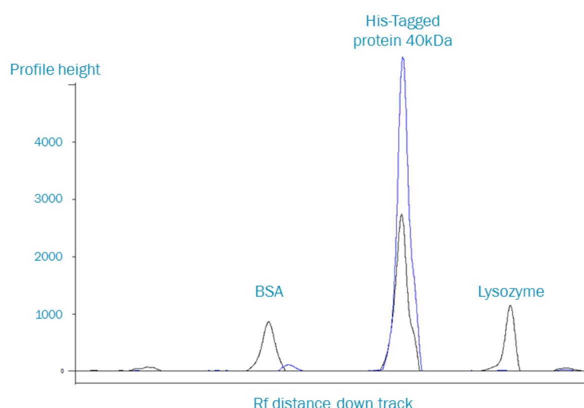

Figure 3: Profile comparison – The contaminated protein sample (His-Tagged samples contaminated with BSA and Lysozyme) is shown as a black line and shows three peaks corresponding to the His-Tagged protein and the two contaminants. In contrast, after purification on the HIS-Select nickel affinity gel (blue line) the product shows a single major band corresponding to the purified 32kDa His-Tagged protein. The relative peak volumes for the 32kDa protein reflect the concentrating effect of the purification.

Benefits of using ScreenTape P200 for monitoring His-Tagged proteins during purification.

- **A more efficient workflow** - The P200 platform streamlines protein fraction QC checks during your His-Tag affinity purification workflow. At less than one minute per sample it is more efficient than current SDS-PAGE methods. The protein electrophoresis bottleneck is therefore eliminated, allowing you to focus on the next stage of your experiment.
- **A safe electrophoresis method** - P200 reagents are pre-packaged and self-contained avoiding messy manual interventions or exposure to dangerous chemicals.
- **Improved reproducibility and accuracy** - Automation and pre-packaged reagents mean ScreenTape is more accurate and reproducible than traditional SDS-PAGE methods, which can vary significantly according to the staining and destaining protocol. Results from several different analyses or batches can therefore be accurately compared.
- **GLP compliant results** - Lab901's integrated GeneTools software makes data analysis, archiving and retrieval simple. Produce GLP compliant reports with one mouse click.
- **Cost effective protein analysis** - Unused lanes on ScreenTape can be used at a later date thanks to a barcode that is unique to every tape. There is no need for samples to be batched to fill every position on a piece of ScreenTape.

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

Lab901, the Lab901 logo, ScreenTape, TapeStation and "More time for discovery" are either registered trademarks or trademarks of Lab901 Limited in the United Kingdom and/or other countries. All other trademarks are the property of their respective owners. ©2009 Lab901 Limited. All rights reserved. GeneTools is a registered trademark of Synoptics Ltd. Protein standards in this product are used under license from Fermentas UAB. Protein stains in this product are used under license from Active Motif Chromeon GmbH.

Lab901 Ltd, IMEX Business Centre, Loanhead, EH20 9LZ, UK
t +44(0) 131 448 2904 **f** +44 (0) 131 448 0679 **w** www.lab901.com