Optimization and control of protein purification procedures with P200 ScreenTape

Application Note

Proteomics

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Abstract

The Agilent 2200 TapeStation instrument and the P200 ScreenTape consumable provide an automated electrophoresis analysis system for proteins of 10 to 200 kDa over a broad range of concentrations and sample buffers for the optimization and control of protein purification. This Application Note describes the applicability of the P200 ScreenTape system for:

- fully automated electrophoretic analysis of IEX fractions
- screening of protein fractions from His-tag affinity purifications
- analysis of column fractions from MBP-tag purifications
- monitoring and optimization of the efficiency of the GST-tag cleavage reaction

P200 ScreenTape simplifies protein analysis because it is easy to use and integrates complete sample analysis. It automatically resolves and annotates protein peaks, allowing efficient and straightforward fraction selection. P200 ScreenTape is fully compatible with commonly used buffer systems allowing the user to load samples directly for rapid analysis. With P200 ScreenTape, 16 protein samples are analyzed in less than one minute per sample. Shortening the time to obtain results on intermediate protein QC checks, allows the study of complex protein fractions and accurate optimization of protein purification or cleavage conditions.

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Introduction

There are several approaches to protein purification intending to isolate a specific protein from a complex mixture, which are based differences in protein size, physical or chemical properties, binding affinity and biological activity. Usually a protein purification protocol contains one or more chromatographic steps. Ion exchange chromatography (IEX), for example, separates proteins according to charge and is commonly used as a first-pass purification protocol for highly complex protein samples such as cell lysates and tissue extracts. Consequently, IEX fractions often contain complex protein mixtures in 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.

The expression of recombinant proteins allows for additional peptide sequences to be added to the protein. The most common application is the addition of sequences, or tags, which can assist in the purification process. These additional sequences are usually added at either the N- or the C-terminus of the protein with the aim of maintaining protein functionality. Poly-histidines are commonly used to tag recombinant proteins as a means to achieve rapid product isolation through affinity purification on metal-ion columns. Another commonly used protein tag is the maltose-binding protein (MBP). It has a high affinity for amylose resin, which makes it possible to achieve protein purification in a single step. MBP-tags are most useful when the target protein is difficult to solubilize. Furthermore, glutathione S transferase (GST) is a 24 kDa epitope tag that is commonly engineered onto recombinant proteins. Glutathione transferases bind glutathione with high affinity and specificity, allowing glutathione based affinity resins to successfully purify GST-tagged proteins.

During the purification process, it is often required to test collected fractions for protein content, expression levels and molecular weights using an electrophoresis method. The most general method to monitor the purification process is by running a conventional SDS-PAGE slab gel. However, this method only gives a rough measure of the amounts of different proteins in the mixture.

In this Application Note, we demonstrate that the P200 ScreenTape analysis is compatible with typical protein purification processes and provides accurate and reproducible performance for rapid and automated analysis of protein purification fractions. First, we show a typical example of IEX for protein purification. Then, the applicability to His- and MPD-tag protein purification is demonstrated. Furthermore, P200 ScreenTape can also be used to optimize and reliably monitor the GST cleavage reaction. The fully integrated analysis includes peak annotation, accurate molecular weight determination, and percent purity values, making the 2200 TapeStation the ideal system for protein purification analysis.

Experimental

Material

Zeba Micro Spin Columns were ordered from Pierce/Thermo Fischer Scientific (Rockford, IL, USA); amylose resin from New England Biolabs (Ipswich, MA, USA); HIS-Select Nickel Affinity Gel, β-galactosidase and lysozyme from Sigma-Aldrich (St. Louis, MO, USA); HiTrap Heparin HP column (cation exchanger), Source 15Q column (anion exchanger), Glutathione Sepharose 4B and PreScission Protease from GE Healthcare (Chalfont St. Giles, UK). The MBP fusion protein was kindly provided by the Division of Signal Transduction Therapy, University of Dundee (Dundee, Scotland, UK). NuPAGE Novex 4-12% Bis-Tris gel, NuPAGE MES SDS Running buffer, Colloidal Blue Staining kit, and SeeBlue Plus 2 Protein Standard were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA); 2200 TapeStation System, P200 ScreenTape and P200 Reagents were obtained from Agilent Technologies, Inc. (Waldbronn, Germany).

Ion exchange chromatography (IEX)

Desalted rat muscle extract was fractionated by anion and cation exchange chromatography. A 4-mL extract containing 22 mg of protein was loaded onto a 1-mL cation exchanger column. The column was washed with 8 mL of low salt buffer (30 mM MOPS pH 6.9, 5% v/v glycerol, 0.03% Brij 35, 7 mM 2-mercaptoethanol). Proteins were eluted in 17 one-milliliter fractions using a 0 to 1.2 M NaCl gradient. The flow through and low salt wash from the initial loading (12 mL at 0.4 mg/mL), was pH-adjusted to 8.2 with NaOH and applied to a 1 mL anion exchanger column. The column was washed with 8 mL of low salt buffer (30 mM Tris pH 8.2, 5% v/v glycerol, 0.03% Brij 35, 7 mM 2-mercaptoethanol). Proteins were eluted in seventeen 0.59-mL fractions using a 0 to 1 M NaCl gradient.

His-tagged protein purification

The His-tag purification method used a nickel affinity gel in phosphate buffer (50 mM NaH_2PO_4, 0.3 M NaCl, pH 8) with concentrations of 10 mM imidazole in the wash buffer, and 250 mM imidazole in the elution buffer. A His-tagged protein of 40 kDa was prepared at 6.5 mg/mL in 25 mM Hepes at pH 7.5, 1 mM DTT, 50% glycerol, and 1 mM benzamidine. Several washes were used to equilibrate the nickel affinity gel into wash buffer. Three 200-µL aliquots of equilibrated gel were re-suspended in either 100 µL of diluted His-tagged protein at 1.3 mg/mL in wash buffer (original protein sample), 100 µL of bovine serum albumin (BSA)/lysozyme mix at 1 mg/mL in wash buffer (the spike), or 100 µL of BSA/lysozyme 1 mg/mL, His-tagged protein 1.3 mg/mL in wash buffer (contaminated protein sample). These gel-protein suspensions were incubated on ice for 30 minutes. Before the elution phase the gels were washed five times in wash buffer. Proteins were eluted from the nickel resin in 100 µL of elution buffer.

MBP-tagged protein purification

A 50-µL sample containing 2 mg/mL MBP fusion protein (89 kDa) and two contaminants: 0.1 mg/mL β-galactosidase (116 kDa) and 0.05 mg/mL lysozyme (14 kDa), in wash buffer (20 mM Tris pH 7.4, 200 mM NaCl, 1 mM EDTA, 1 mM DTT) was added to a microspin column containing 25 µL of prepared amylose matrix and incubated on ice for 30 minutes. Following affinity binding, the mobile phase was collected in a single fraction by centrifugation to recover the unbound proteins. Removal of contaminating proteins was carried out by washing the column seven times by centrifugation after addition of 1 mL of wash buffer, generating seven fractions. Three 25-µL volumes of elution buffer (20 mM Tris pH 7.4, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM maltose) were used to elute the MBP fusion protein from the amylose column.

GST-tag cleavage from purified GST fusion protein

A GST-tagged protein of 130 kDa at 0.4 µg/µL was cleaved overnight at 8 °C on a glutathione sepharose matrix in a micro spin column using 8 mU/µL protease. The digestion mixture was incubated with glutathione sepharose for 30 minutes on ice and then loaded onto the microspin column. The protease and free GST were immobilized, leaving the protein of interest free in solution. Following centrifugation and two wash and spin steps with glutathione free buffer, the protein was collected into three fractions. Two wash and spin steps in elution buffer containing 20 mM reduced glutathione were used to regenerate the column, resulting in two fractions containing the eluted GST and protease.

P200 ScreenTape analysis procedure

All samples were pre-stained and prepared for P200 ScreenTape analysis following the standard method. Pre-staining during the sample preparation stage avoids lengthy staining and destaining procedures that are common to conventional SDS-PAGE methods. The prepared samples were placed in the 2200 TapeStation instrument with P200 ScreenTape and tips. The analysis was started from the TapeStation software; full analysis of the samples was achieved and archived, with no user intervention, in less than one minute per sample.

SDS-PAGE

For comparison, His-tagged protein samples were also prepared for analysis on a 4-12% SDS-PAGE gel with 2-(N-morpholino)ethane sulfonic acid (MES) running buffer and stained using Coomassie blue, following the manufacturer's instructions.

Results and Discussion

IEX fraction selection with P200 ScreenTape

A desalted rat muscle extract was fractionated by anion and cation exchange chromatography. A 2-µL aliquot from each of the collected fractions was analyzed on P200 ScreenTape to demonstrate the suitability of the system. The 2200 TapeStation software displays fully analyzed results, which included a familiar gel image, an electropherogram and a table with protein molecular weights and purity. Figure 1 shows that each lane contained internal markers (green) that allow reproducible protein sizing. The automatically obtained data allows users to compare sample compositions and easily determine the percent gradient for target-protein elution (Figure 1). The sensitivity and wide quantitative range of P200 ScreenTape delivered accurate results for complex IEX fractions.

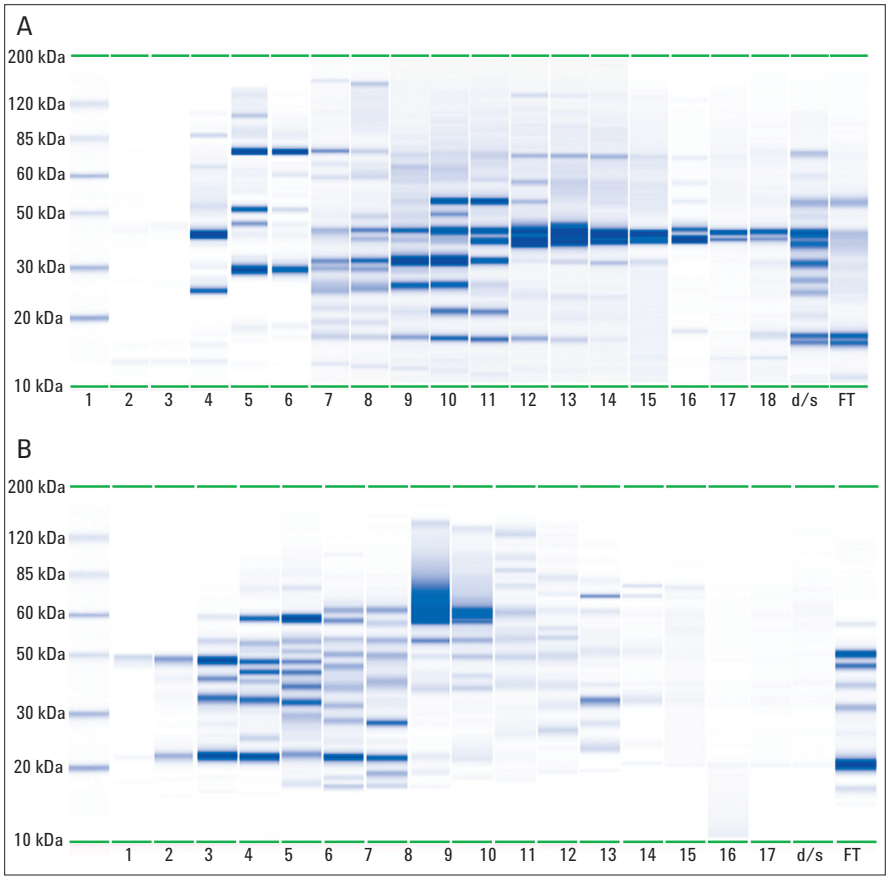

Figure 1
P200 ScreenTape gel images from anion (A) and cation (B) exchanger fractions. Cation exchanger samples were diluted 1:5 prior to analysis. Column flow through (FT) and low salt wash (d/s) from the anion exchanger were combined to form the starting material for the cation exchange purification. The P200 ladder is shown in the first lane, internal markers are highlighted in green.

His-tag affinity purification

A His-tagged protein of 40 kDa was affinity purified and then analyzed using P200 ScreenTape and SDS-PAGE for comparison (Figure 2). Fractions from nickel matrix purifications were used directly on P200 ScreenTape, even though they contained imidazole concentrations of up to 250 mM. This buffer tolerance avoided an extra buffer exchange step prior to sample preparation. The His-tagged protein contaminated with BSA and lysozyme showed as expected three main bands. After purification, the fraction showed a single major band corresponding to the purified His-tagged protein. The relative peak purity for the His-tagged protein reflected the concentrating effect of the purification. P200 ScreenTape demonstrated accurate and reproducible performance for monitoring of His-tag purification. Proteins were accurately sized, while impurities were detected, sized, and quantified.

For comparison, the results from the same samples run on SDS-PAGE are shown in Figure 2B. These were obtained after three hours of manipulation with several time-consuming steps, which included buffer preparation, manual gel loading, staining, and destaining. P200 ScreenTape results were available in less than one minute per sample and the data was automatically presented and archived.

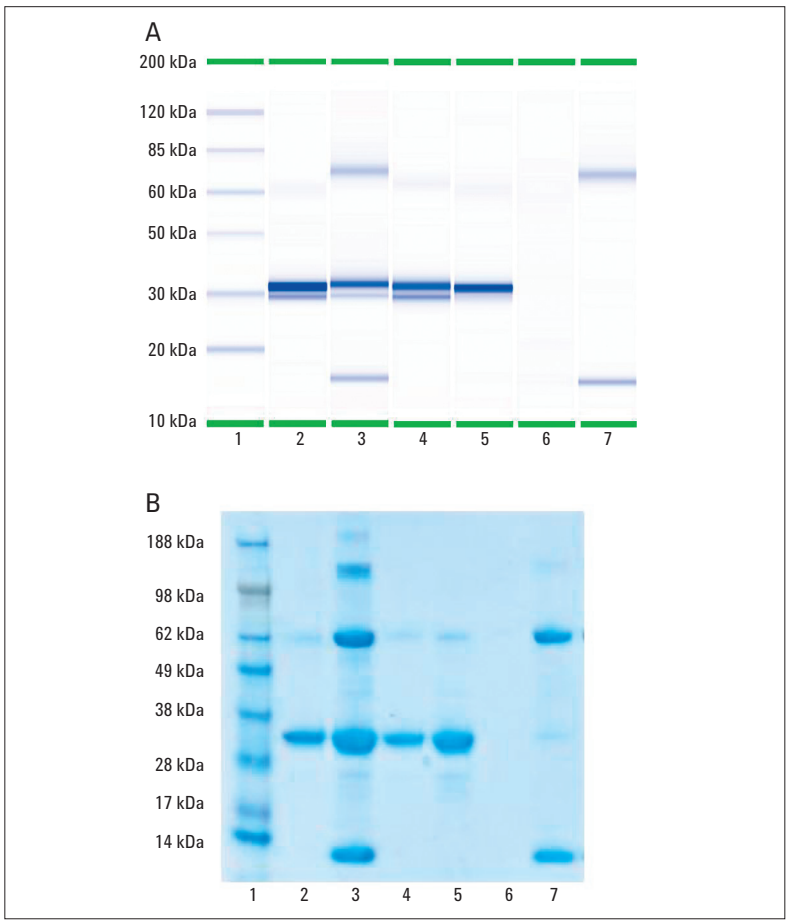

Figure 2
Analysis of fractions from His-tagged protein purification with the P200 ScreenTape (A) and SDS-PAGE stained with Coomassie (B). Lane 1 (A) - P200 ladder, lane 1 (B) – SDS-PAGE ladder, lane 2 - original sample, lane 3 - contaminated sample, lane 4 - contaminated sample after purification, lane 5 - original sample after purification, lane 6 - spike after purification, and lane 7 - spike.

MBP-tagged protein purification

Figure 3 demonstrates that P200 ScreenTape accurately separated and sized a MBP-tagged protein in fractions collected from amylose affinity purification. The fraction contained salt concentrations of up to 200 mM and additives such as maltose, DTT, and EDTA. Figure 3A shows the analysis of different samples obtained during the purification of the MBP-tagged protein (sized at 89 kDa) contaminated with β-galactosidase (sized at 121 kDa) and lysozyme (sized at 15 kDa), as well unidentified contaminants at 77 and 66 kDa. Lanes 4 and 5 show two elution fractions containing the target protein. Electropherograms from different samples were overlaid for direct peak comparison. The overlay function of the 2200 TapeStation software (Figure 3B) makes it simple to see differences in protein levels and types of impurities. P200 ScreenTape demonstrated both accurate and reproducible performance for protein fraction analysis. Purified MBP-tagged proteins were accurately sized, while impurities were precisely detected, sized, and quantified.

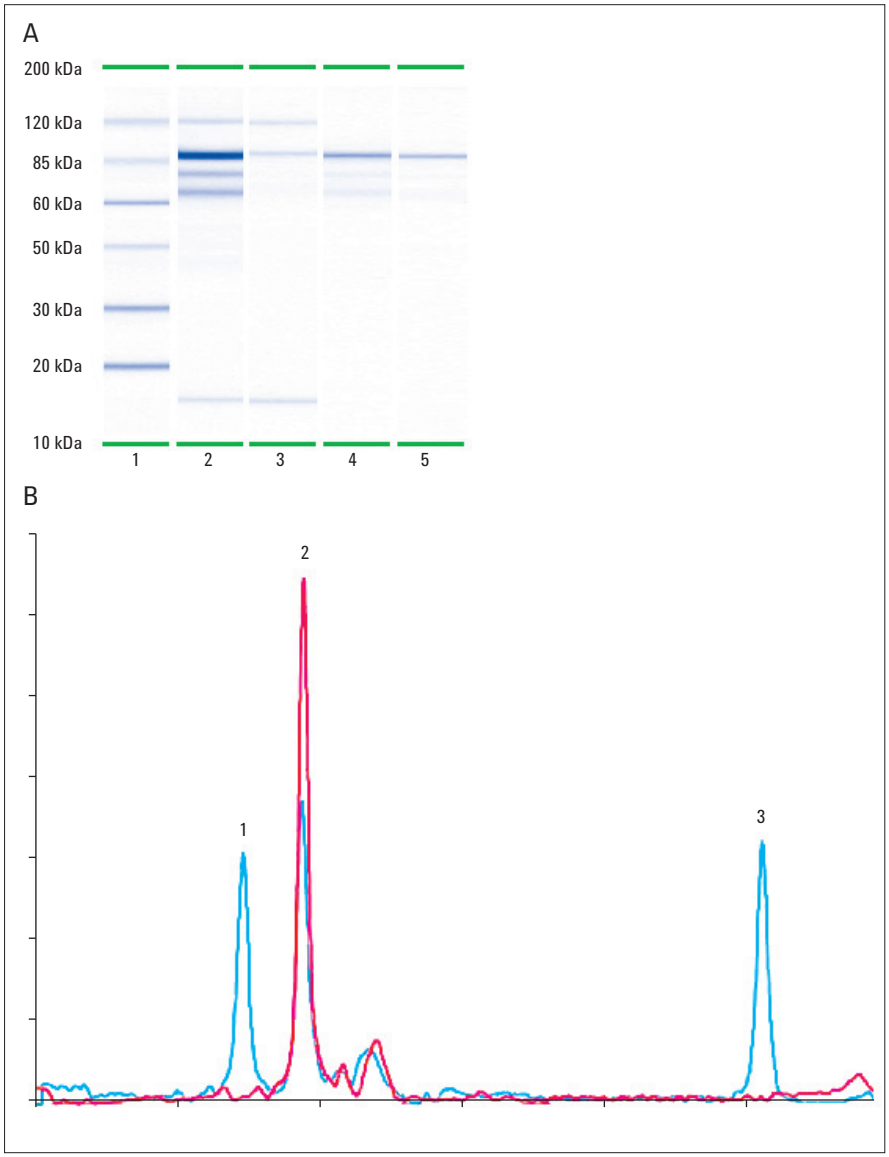

Figure 3
Analysis of amylose affinity gel fractions with P200 ScreenTape. Gel image (A): lane 1 - P200 ladder, lane 2- MBP-tagged protein in the presence of contaminants, lane 3 - wash 1, lanes 4 and 5 - elution fractions 1 and 2. Electropherogram overlay (B): blue trace - wash 1, red trace - purified protein, peak 1 - β-galactosidase, peak 2 - MBP-fusion protein, peak 3 - lysozyme.

GST-tag cleavage from purified GST fusion protein

Figure 4A illustrates the analysis of a GST-tagged protein during cleavage. P200 ScreenTape was used to check for fusion protein tag removal. It was confirmed by comparing the molecular weight of the cleaved protein in lane 2 (143 kDa) with that of the GST-tagged protein in lane 9 (167 kDa). This shift corresponded to the removal of the GST-tag. Free GST (24 kDa) and protease (46 kDa) were also observed. The P200 ScreenTape was also used to monitor column regeneration after glutathione elution.

The molecular weight shift of 24 kDa in the target protein before and after GST-tag removal was also observed using the overlay function of the TapeStation software (Figure 4B). The protein preparation also contained several other contaminating proteins.

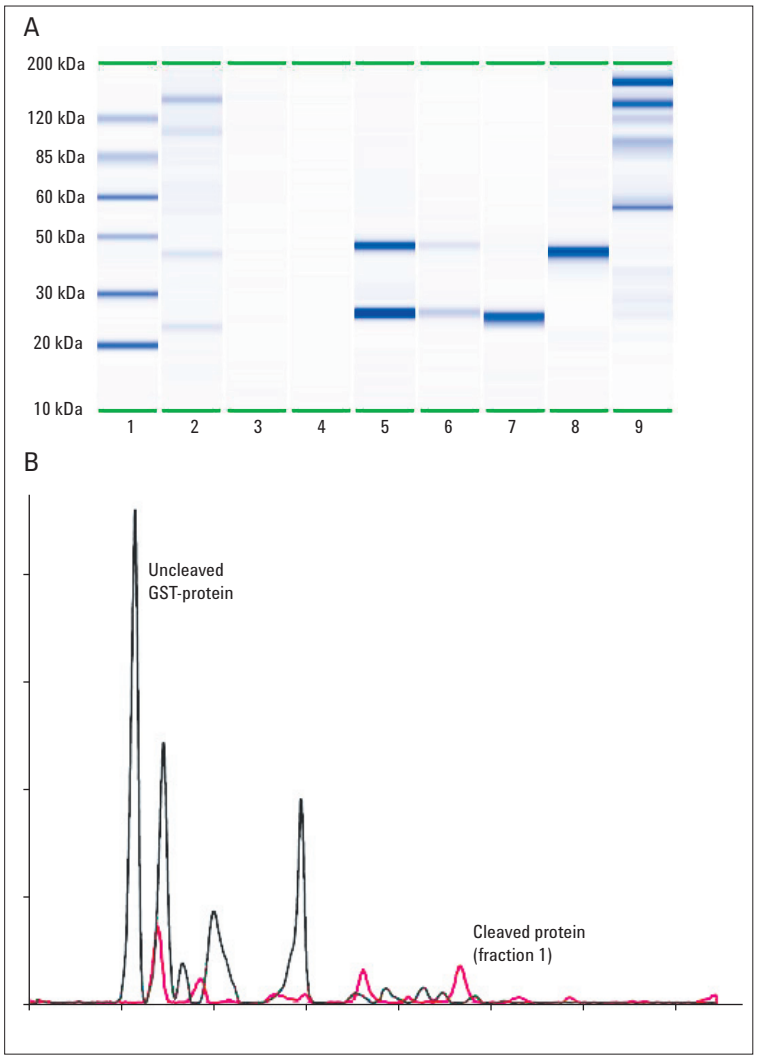

Figure 4
Analysis of GST cleavage from a fusion protein on P200 ScreenTape. Panel A, lane 1 – P200 ladder, lanes 2 to 4 – cleaved protein, lane 5 and 6 – eluted GST and protease, lane 7 – GST, lane 8 – protease, lane 9 - uncleaved GST protein. Panel B – overlay of electropherograms from lane 2 and 9 showing the target protein before (black trace) and after GST-tag cleavage (red trace).

Conclusion

P200 ScreenTape demonstrated accurate and reproducible performance for protein purification analysis and monitoring protein tag removal. The target proteins were accurately sized, while impurities were detected and sized.

P200 ScreenTape for protein purification analysis provides:

- Fast and reliable protein fraction control during the purification workflow, allowing users to select relevant fractions more easily and to proceed with downstream applications more quickly.
- Improved reproducibility and accuracy due to pre-packaged reagents, full automation, and standardized methods giving better confidence when checking for protein purity or tag removal.
- A versatile method suitable for many different protein purification analysis tasks due to the sensitivity, dynamic range, and buffer compatibility.
- Report format for electrophoresis results, which enables easy batch to batch comparison and accurate record keeping.

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