Understanding the Effects of Proteins and Buffers on Staining, Denaturation, and Electrophoresis when Analyzing Proteins with Agilent P200 ScreenTape

Technical Overview

Introduction

Protein preparation and purification methods use large varieties of buffers, salts and other additives that span a broad range of concentrations and pH values. Proteins themselves are also highly variable due to, for example, amino acid composition, modifications, and structure. This variability in proteins and buffers requires some consideration when handling and analyzing proteins. Protein samples are typically analyzed using SDS-PAGE, capillary electrophoresis (CE), on-chip electrophoresis with the Agilent 2100 Bioanalyzer system, or automated electrophoresis with the Agilent 2200 TapeStation system. Protein properties and buffer components can have unanticipated impacts when transferring methods from one analysis system to another.

Agilent P200 ScreenTape and the 2200 TapeStation system are compatible with the majority of proteins and buffers that are commonly used for protein purification and preparation. This Technical Overview provides some background on the effects that can be observed when analyzing certain proteins samples. In addition, it lists buffer components that are compatible and outlines considerations that should be taken for other buffer components when using P200 ScreenTape.

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Sample Handling

Sample preparation before analysis with P200 ScreenTape includes two main steps; the staining reaction, and denaturation (Figure 1), both steps are carried out at 75 °C. For consistent and reproducible results, it is necessary to consider the following:

- Agilent P200 sample buffers and ladder contain sodium dodecyl sulfate (SDS). Remove reagents from the freezer and ensure that they are thoroughly equilibrated to room temperature prior to use, to avoid precipitation.
- Storing the Agilent P200 ladder and Agilent P200 sample buffers on ice after thawing can cause precipitation.
- Once equilibrated, thoroughly mix the reagents by vortexing and then centrifuge briefly at 500 rpm to remove any material from the lids.

Impact of Protein Properties on Staining Reaction

Proteins are stained prior to electrophoresis with the 2200 TapeStation system. The Agilent P200 labelling dye used in this reaction is a reactive florescent dye that covalently binds to the primary amines of both lysine side chains and the N-terminus of a protein. This reaction requires not only the presence of these primary amines in the protein, but also their availability to react with the dye. The labelling efficiency might be decreased for all proteins, or only for certain proteins depending on the cause:

- Modifications to either the N-terminus or the lysine side chains will reduce staining.
- Modifications, not targeted at the primary amine side chains, such as pegylation of cystein residues can reduce lysine accessibility.
- Insufficient cell lysis or solubilization prior to the nondenaturing staining reaction affects the overall signal intensity.

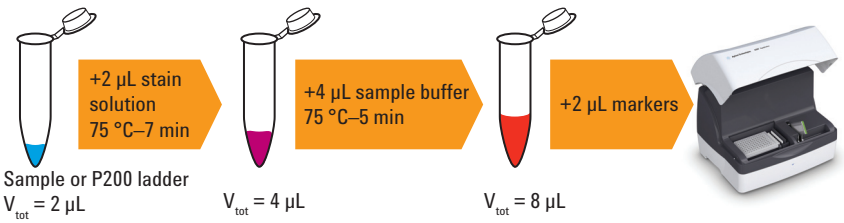

Figure 1. P200 ScreenTape sample preparation.

For example, some commercial protein ladder preparations demonstrate very low staining efficiencies, because the ladder proteins are highly modified so that the P200 labelling dye cannot react with the protein. Plus, these ladder preparations often contain high levels of medium to low compatibility additives such as SDS (Table 1).

Influence of pH on Staining

The staining reaction occurs optimally between pH 8–10 as the amine group needs to be deprotonated to be amenable to the dye. In most cases, the P200 labelling buffer provides sufficient buffering capacity. However, certain applications at extremely low pH, or with high buffering capacity at moderate pH, may prevent the optimal pH range for the staining reaction being achieved. To address staining issues where pH is implicated, P200 pH buffer, buffered around pH 9, is supplied with the P200 reagents (5067-5372). Use the P200 pH buffer to dilute the protein sample to an appropriate level to mitigate the effects of low pH on staining efficiency.

The use of P200 pH buffer as a general dilution buffer is not recommended as the combination of several buffer components can sometimes lead to unexpected results.

Denaturation

The denaturation step in P200 sample preparation is essentially the same as denaturation for standard SDS-PAGE slab gels. Therefore, there are similar considerations at this stage with regard to buffer components:

- Guanidine, for example, has a relatively low compatibility at 600 mM, caused by SDS precipitation (Table 1). The alternative, urea, is fully compatible at concentrations up to 8 M.
- Certain heat tolerant proteases are not sufficiently inactivated by the denaturing step, resulting in sample and marker degradation. Therefore, protease inactivation prior to sample preparation is recommended.

Sample Conductivity Affects Migration

Due to the reduced dimensions of the P200 ScreenTape and the individual nature of the lanes, sample conductivity can contribute towards electrophoretic migration. The increase in sample conductivity manifests itself by retardation of the so called dye front beyond which protein separation is not possible. The retardation of this front becomes an issue when the salt concentration is above 500 mM, where the front begins to retard the internal 10 kDa marker. Higher salt levels (>1 M) are compatible with P200 ScreenTape, but sizing accuracy can be affected. Sizing issues caused in this manner can be somewhat compensated for in the 2200 TapeStation software by unaligning the image to check the bottom marker is being overly retarded. If this is the case, the default sizing settings should be changed from *Between Markers* to *From Position* in the *Molecular Weight Settings* option in the *Calibration* tab of the software. Please be aware that smaller proteins may begin to comigrate with the 10 kDa bottom marker affecting sizing. More generally, sizing reproducibility may also be compromised when these settings are applied.

Table 1 outlines the known compatibility issues for common buffer components and additives. The first response to any buffer compatibility issue should be to dilute the sample below the stated tolerated concentration. Dilution with water is suitable in most cases, but other buffers like phosphate buffered saline (PBS) have successfully been employed. Other techniques such as buffer exchange by dialysis or using desalting spin columns have also shown positive results.

Table 1. Compatibility of P200 ScreenTape with sample buffers and buffer components.

Buffer or component	Compatibility at normal working concentration	For best results use less than	Recommendations and tips
Common buffers			
Phosphate	High		
Citrate	High		
Acetate	High		
HEPES	High		
MES	High		
MOPS	High		
Salts			
NaCl	Medium	500 mM	High levels of salt lowers the effective charge of the sample, retarding migration and affecting MW accuracy. • If possible dilute sample. • Analyze ladder and sample in the same buffer composition. • Desalt sample.
Potassium	Low		
Urea	High	8 M	
Reducing agents			
DTT	High		
β-mercaptoethanol	High		
Detergents			
Tween	High		
NP-40	High		
Brij 35	High	0.3 %	The supplied reagents contain sufficient SDS for analysis, additional SDS can reduce assay sensitivity.
SDS	Medium	1 %	High levels of Triton can cause an issue with reduced staining and profile retardation. Dilution with water resolved this issue.
Triton X-100	Medium		
Sugars			
Sucrose	High		High viscosity (see Glycerol)
Trehalose	High		High viscosity (see Glycerol)
Solvents			
Acetonitrile	Medium	20 %	Care must be taken when analyzing higher concentrations to avoid evaporation during heating.
Methanol	High	20 %	
Ethanol	High	20 %	
DMSO	High		
DMF	High		
Amines			
Primary (for example Tris or glycine)	Medium	50 mM	Primary amines interfere with the staining reaction and reduce sensitivity, but not sizing. • Include an internal standard during quantification. • Buffer exchange samples when sensitivity is important.
Secondary	Medium	100 mM	Secondary amines may interfere with staining (see primary amines).
Tertiary	High		
Chaotropic agent			
Guanidine	Low	600 mM	Guanidine causes the precipitation of SDS and has a strong detrimental effect upon the assay. • If possible dilute guanidine to < 600 mM. • Vortex samples well during sample preparation as guanidine can cause protein to precipitate after heating. • Preferably use urea, or where possible, buffer exchange into a guanidine free solution.
Other			
Glutathione	High	20 mM	
Glycerol	High		Care must be taken when pipetting small volumes of high viscosity samples to ensure reproducibility of analysis.
Low pH			Acidic samples with strong buffering capacity may require dilution or pH adjustment before analysis.

Conclusion

The Agilent P200 ScreenTape with the Agilent 2200 TapeStation system accurately sizes and analyses protein samples. However, it is important to understand that certain protein properties or sample buffer components can affect the performance of the P200 ScreenTape staining reaction, the denaturation step or electrophoresis more generally.

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