

Reproducible integrity and purity testing of antibodies with P200 ScreenTape

Application Note

Proteomics

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Abstract

The Agilent 2200 TapeStation instrument and P200 ScreenTape consumables provide an automated electrophoresis analysis system for proteins of 10 to 200 kDa. P200 ScreenTape automates the analysis of monoclonal antibody (mAb) samples under reducing and non-reducing conditions. Integrity and purity analysis of up to 16 mAb preparations can be performed in less than one minute per sample without any manual intervention. The ability of P200 ScreenTape to run as few as two samples at a time without sacrificing the remaining lanes is also ideally suited to continuous monitoring directly from the culture. This level of automation makes manufacturing quality control procedures for mAbs more efficient, more cost effective and less labor intensive. Electropherogram and gel images and other relevant data are automatically generated and archived and can easily be retrieved and printed.

Introduction

Monoclonal antibodies (mAbs) are an essential tool in the life sciences and are used in many cutting-edge applications such as antibody therapy, diagnostic testing, and protein microarray analysis. mAbs are also one of the fastest growing areas of novel drug development within the pharmaceutical industry. Stringent testing of mAbs throughout production is required, with in-process quality control and stability testing forming a large part of the manufacturing process. As demonstrated in this Application Note, P200 ScreenTape provides the necessary reliability and speed of analysis for product quality measurements because the platform is automated and delivers results for 16 samples in less than one minute per sample. P200 ScreenTape offers a more accurate, precise, and convenient method for mAb analysis than traditional SDS-PAGE methods, as both reducing and non-reducing analysis can be run side-by-side and as few as two samples can be analyzed without sacrificing unused lanes.



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Experimental

Material

NuPAGE Novex 4-12% Bis-Tris gel, NuPAGE MES SDS Running buffer, Colloidal Blue Staining kit, Silver Staining kit, and See Blue 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 (Waldbronn, Germany).

P200 ScreenTape analysis procedure

Formulated mAb samples, diluted to 0.5 mg/mL with distilled water, were prepared for P200 ScreenTape analysis following the standard method. The pre-staining avoids lengthy staining and destaining procedures that are common to conventional SDS-PAGE methods. The availability of P200 Reducing or P200 Non-Reducing Sample buffer within the P200 Reagents kit allows for analysis of samples under both reducing and non-reducing conditions. The prepared samples were placed in the 2200 TapeStation instrument with P200 ScreenTape and tips. The analysis was started from the 2200 TapeStation software; full analysis of the samples was achieved and archived, with no user intervention, in less than one minute per sample.

SDS-PAGE

The same samples were also run on a 4-12% SDS-PAGE gel under reducing and non-reducing conditions with 2-(N-morpholino)ethane sulfonic acid (MES) running buffer and stained using Coomassie blue or silver stain, following the manufacturer's instructions.

Results and Discussion

QC of mAbs resolved in reducing and non-reducing conditions on P200 ScreenTape

The 2200 TapeStation software automatically presented analyzed protein results for mAb samples, the gel image displayed in Figure 1A. Results are presented either as an electropherogram or the more familiar gel image. A table is also presented containing information on protein molecular weights and purities. Peaks are automatically located and can easily be compared between samples using the overlay function in the comparison mode, so that the different levels of impurities between preparations are obvious. The three different mAb samples that were run in reducing conditions (lanes 2 to 4) showed major peaks at 48 and 28 kDa corresponding to mAb heavy and light chains. The analysis of the same mAb preparations under non-reducing conditions (lanes 5 to 7) showed a major peak at 120 kDa (whole Ig) and smaller bands at 93, 76, 59, 48, and 28 kDa corresponding to various heavy/light chain combinations. This analysis showed for example that the whole Ig sample, shown in lane 5, is only 57% pure. The abundance of these additional bands may be caused by an auto-reduction reaction whereby the heating of the mAb with SDS exposes cryptic cysteines. These exposed cysteines can cause a low level of reduction leading to the appearance of partially reduced mAb fragments¹.

Similar separations were observed with the mAb samples when run on SDS-PAGE gels Coomassie or silver stained only after 3 to 5 hours, which included buffer preparation, manual gel loading, staining and destaining (Figure 1 B and C). Further, these results were not automatically archived on a gel documentation system for any sizing or peak purity values.

The staining and destaining methods common to SDS-PAGE can introduce a lot of variability in mAb QC results. This lack of consistency does not occur on P200 ScreenTape as all the reagents are pre-packaged into kits delivering excellent reproducibility. Further, P200 ScreenTape does not suffer from diffusion of reducing agents between gel lanes as the samples migrate in separate channels. On slab gels this problem can often lead to erroneous results and may force users to leave empty lanes between reduced and non-reduced samples (as was needed in the SDS-PAGE gels presented here in Figure 1B and C).

Taken together, these results demonstrate that P200 ScreenTape is a method of choice for separating and sizing mAbs during in-process QC and stability testing. Reproducible electrophoretic separations are not only achieved but also fully annotated in less time and with fewer labor intensive steps than with traditional slab gel methods. In addition, the data can be automatically archived and printed.

Linearity of protein migration according to molecular weight on P200 ScreenTape

P200 ladder, containing molecular weight standard proteins of 120, 85, 60, 50, 30, and 20 kDa, was run on three different P200 ScreenTape. Following electrophoresis and analysis, final peak positions for the standards, including the internal markers at 10 and 200 kDa, were plotted against the logarithm of their molecular weight. Linear regression analysis was performed on this data, and the resulting trend line was used to back-calculate the molecular weight of known standards. Results in Figure 2 show an excellent correlation between the protein peak positions and protein molecular weights ($R^2 > 0.98$ for all P200 ScreenTape studied). Consequently, P200 ScreenTape is an exact method for sizing proteins, with a separation profile similar to a

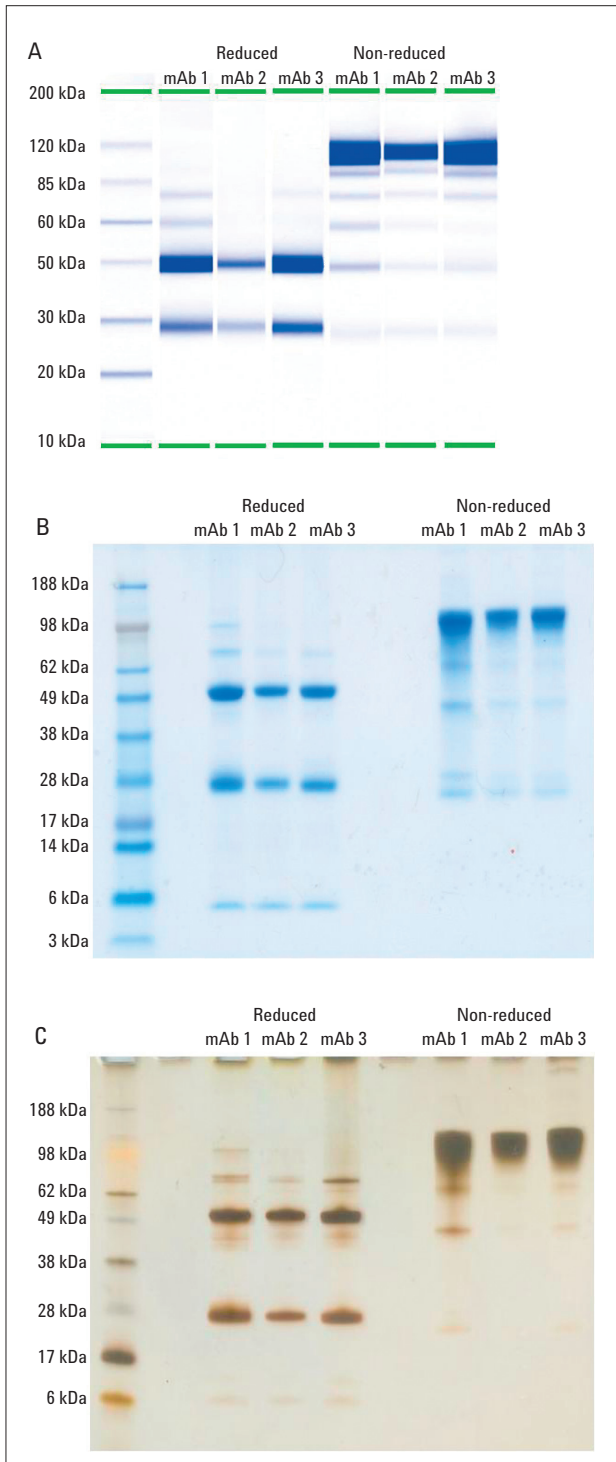


Figure 1
Separation of three different mAb preparations with the P200 ScreenTape (A), or 4-12% SDS-PAGE gel stained with Coomassie blue (B) or with silver stain (C) in reducing and non-reducing conditions. Lane 1 contains the P200 ladder (A) or a SDS-PAGE ladder (B and C). Green bands in each lane are internal P200 markers.

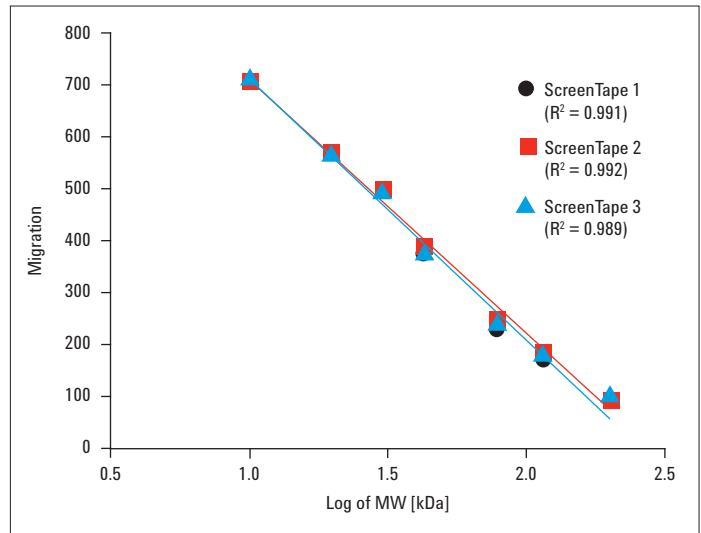


Figure 2
Graph showing the linear migration of proteins according to molecular weight from three different P200 ScreenTape.

4-12% gradient SDS-PAGE. Further analysis of this data shows that sizing accuracy for proteins between 10 and 200 kDa is $\pm 8\%$. This offers significant improvements on traditional slab gel performance, where sizing accuracy is typically $> 15\%$.

Precision of protein sizing on P200 ScreenTape

Three preparations of mAb samples at 0.5 mg/mL were loaded in reducing and non-reducing conditions on three different P200 ScreenTape. Heavy and light chains were automatically sized and their purity determined. Mean values and CVs were calculated for the molecular weights and peak volumes returned by the 2200 TapeStation software.

Results in Table 1 show that protein sizing on P200 ScreenTape is precise to $\pm 1\%$ for reduced mAb samples, and protein purity determination is precise to $\pm 5\%$ for the mAb heavy chain and $\pm 9\%$ for the mAb light chain. Consequently, P200 ScreenTape is a method of choice for mAb testing as it offers highly reproducible sizing for immunoglobulin fragments and a precise platform for mAb purity testing.

ScreenTape	Molecular weight (kDa)		Purity (%)	
	Heavy chain	Light chain	Heavy chain	Light chain
1	42.5	29.7	62.0	38.0
	42.7	30.1	63.2	38.0
	43.0	30.0	62.7	37.4
2	42.5	29.4	63.2	36.8
	42.3	29.7	64.5	35.5
	42.3	29.4	72.1	27.9
3	42.5	29.5	63.1	36.9
	42.6	30.0	64.0	36.0
	42.7	29.9	63.7	36.3
Average	42.6	29.7	64.3	35.7
Standard deviation	0.2	0.3	3.0	3.0
CV (%)	0.51	0.90	4.71	8.47

Table 1
Precision of mAb analysis with P200 ScreenTape. The table summarizing the results returned 2200 TapeStation software for protein molecular weight calculation and purity of three replicates of reduced mAb samples run with three different P200 ScreenTape.

Conclusion

The P200 ScreenTape provides many benefits for monitoring mAb preparations during purification and quality control:

- A more efficient QC method compared to current SDS-PAGE methods, providing automation and increased speed of analysis and therefore eliminating the protein electrophoresis bottleneck and allowing better planning through manufacturing.
- The accuracy of protein sizing and reproducibility of purity determination are superior to traditional methods due to pre-packaged reagents, without any complex manual interventions and automated data analysis.
- Through automation, process control and data archiving P200 ScreenTape allows results from several different mAb batches to be accurately compared. The TapeStation software makes it simple to keep manufacturing records up-to-date and enables easy data retrieval.
- Segregation of samples into channels during electrophoresis prevents the diffusion of reducing agents between sample lanes. Individual mAb samples can be run on P200 ScreenTape without sacrificing unused channels, optimizing tape usage and reducing consumable costs making P200 ScreenTape ideal for at-line monitoring of antibody production.

References

1. "Suppression of antibody reduction artifacts with NEM on P200 ScreenTape," Agilent Technologies Technical Overview, publication 5990-8762EN, 2011.

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