Suppression of antibody reduction artifacts with NEM on P200 ScreenTape

Technical Overview

Introduction

Monoclonal antibodies (mAbs) are now an essential tool in life sciences and have found their way into 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 (QC) and stability testing forming a large part of the manufacturing workflow. P200 ScreenTape provides the necessary reliability and speed of analysis for industrial quality assurance (QA) and QC support because the platform is fully automated and delivers results for 16 samples in less than one minute per sample. P200 ScreenTape also offers a more accurate, precise, and convenient method for mAb analysis than traditional SDS-PAGE methods, as reducing and non-reducing analysis can be run side-by-side.

It has been reported that certain subtypes of IgG can have a higher than expected representation of the half antibody, when analyzed under denaturing, non-reducing conditions.[1] For instance, human subtype IgG_4 has been shown as having a particularly high representation of half antibody on SDS-PAGE. Cryptic cysteins, which do not form disulfide linkages, are exposed in the presence of the denaturant dodecyl sulfate causing partial reduction of the antibody.[1] Treatment of the antibody with N-ethylmaleimide (NEM) prior to SDS-PAGE analysis reduced the appearance of the half antibody (HL) by alkylation of the free cystein residues, preventing auto-reduction. This technical overview shows that the treatment of labile IgGs with N-ethylmaleimide reduces the formation of these denaturing artifacts on P200 ScreenTape under non-reducing conditions.

Experimental

Material

N-ethylmaleimide (NEM) was ordered from Sigma-Aldrich (St. Louis, MO, USA); NuPage Novex 4% to 12% Bis-Tris gel and Colloidal Blue Staining kit were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA); 2200 TapeStation System, P200 ScreenTape and P200 Reagents were obtained from Agilent Technologies (Waldbronn, Germany).

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Antibodies

Prior to the protein analysis with the P200 ScreenTape, 2 µL of two mAb samples (0.5 mg/mL in PBS) exhibiting a higher than anticipated presence of reduction products, were preincubated with 1 µL of a fresh preparation of NEM (100 mM stock dissolved in water to a final concentration of 20 mM), for 10 minutes at room temperature. The reactions were carried out at a neutral pH (in PBS) because, at an elevated pH, NEM can react with lysine residues and produce unexpected results.

Antibody analysis with P200 ScreenTape

The NEM treated and untreated mAbs were then stained, denatured, and analyzed on P200 ScreenTape according to the standard non-reducing protocol.

SDS-PAGE

The same mAb samples were run on a 4% to 12% Bis-Tris gel and the bands were visualized with colloidal Coomassie according to the manufacturers' protocol.

Results and Discussion

Monoclonal antibody samples treated with NEM were analyzed alongside untreated controls on P200 ScreenTape (Figure 1). The analysis of the same NEM treated and control mAb samples were performed on a precast 4% to 12% SDS-PAGE gel under non-reducing conditions, the bands were visualized with colloidal Coomassie (Figure 2). A very similar pattern of fragmentation is reproduced on the slab gel as seen on P200 ScreenTape. The reduction in the formation of the artifacts is less clear on the slab gel due to lower sensitivity of colloidal Coomassie compared to P200 ScreenTape fluorescent stain.

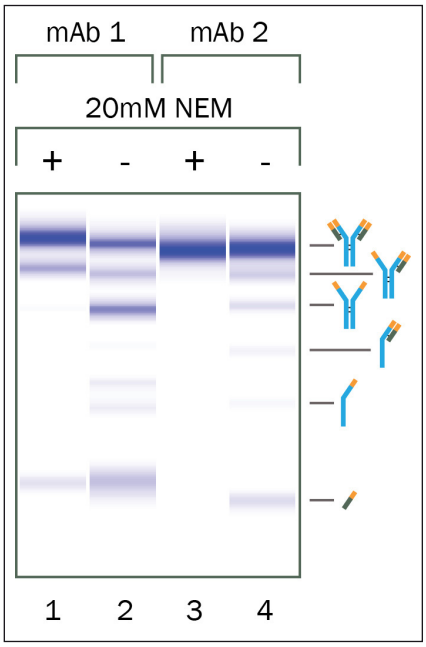

Figure 1
Analysis of two monoclonal IgG samples treated with NEM (+) or untreated (–) on P200 ScreenTape. Schematic representations of the antibody fragments are indicated next to the relevant bands.

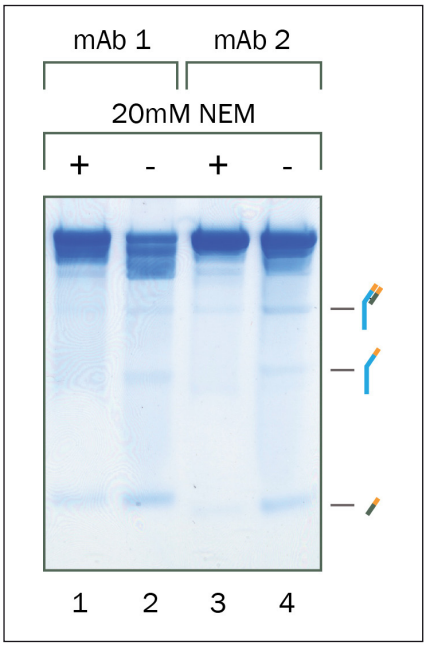

Figure 2
Analysis of two monoclonal IgG samples treated with NEM (+) or untreated (-) on a 4-12% SDS-PAGE gel stained with colloidal Coomassie. Schematic representations of the antibody fragments are indicated next to the relevant band.

Conclusion

The pre-treatment of a monoclonal IgG, susceptible to dodecyl sulfate induced auto-reduction, with NEM reduces the formation of antibody artifacts. This treatment is fully compatible with P200 ScreenTape, which also demonstrates superior peak recognition and limits of detection when compared to colloidal Coomassie based detection. This, in combination with a wider quantitative range, makes P200 ScreenTape ideal for accurate and reproducible quantification of antibody samples.

References

1.
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