

# Analysis of bispecific antibodies using the Agilent 2100 bioanalyzer and the Protein 200 Plus assay

# Introduction

The discovery of hybridoma technology by Kohler and Milstein in 1975 induced a new era in antibody research and clinical development. Nowadays large antibody libraries exist and many new methods have evolved in order to specifically isolate and design antibodies with desired characteristics for unique applications. Engineered antibodies now represent over 30 % of biopharmaceuticals in clinical trials<sup>1</sup>.

Bispecific antibodies contain two different binding specificities fused together and can be used as powerful therapeutic reagents. In cancer therapy, for example, half of the bispecific antibody is engineered to effectively target tumorassociated antigens. The other half of the bispecific antibody can then deliver a cytotoxic payload, such as radionuclides, toxins or immune effector cells (figure 1) to the tumor. The analysis of such antibodies during development, production and purification in terms of quantitation, purity and integrity is more than crucial.

# Application

In this study we show that the Agilent 2100 bioanalyzer together with the Protein 200 Plus LabChip<sup>®</sup> kit is an ideal tool to substitute for the traditional and labor-intensive SDS-PAGE analysis. The disposable Protein 200 Plus chip can analyze 10 protein samples in less than 30 minutes. In addition, the bioanalyzer can perform antibody analysis under both reducing and non-reducing conditions in a single chip run.

# **Experiments and Results**

Bispecific antibody samples were kindly provided from a biopharmaceutical company. Reduced and



#### Figure1

Schematic illustration of the strategic use of bispecific antibodies in cancer therapy Tanja Neumann

non-reduced antibodies were analyzed side by side using the 2100 bioanalyzer. The chip-based separations were performed with the Protein 200 Plus LabChip<sup>®</sup> kit and dedicated Protein 200 Plus assay software. All chips were prepared according to the protocol provided with the kit. The kit includes 25 chips, spin filters and all the reagents necessary for an experiment, including the Protein 200 Plus ladder, as well as the upper and lower marker premixed in the sample buffer.

Figure 2 exemplifies the results obtained. In this case the bispecific antibody consists of two different heavy and light chains stemming from diverse organisms. The gel-like image of the analysis under reduced conditions (figure 2A) shows a clear separation between the sets of two different light and heavy chains. This level of resolution was not achieved using a 4-12 % gradient SDS-PAGE gel (figure 2A). Although the light chains were separated on the SDS-PAGE gel, the two heavy chains were not-they merged into one band.







#### Figure 2

Example of the performed analysis on the Agilent 2100 bioanalyzer using the Protein 200 Plus assay (A) Comparison of the gel-like image (left) and a scanned, Coomassie stained 4-12 % SDS-PAGE gel (right) The numbers indicate the molecular weight in kDa. (B). Overlay of the 2100 bioanalyzer electropherograms of reduced and non-reduced antibody sample is shown.

Figure 2B shows the overlay of the electropherograms of the analysis under reduced and nonreduced conditions. The automated data analysis assigned a size of 159 kDa to the intact antibody. In addition, two minor peaks at 87 kDa and 134 kDa were detected, which represent 5 % of the total sample amount. The reduced antibody analysis revealed two light chain peaks at 23 kDa and 30 kDa and two heavy chain peaks at 57 kDa and 62 kDa. The absence of the larger peaks indicates that they may consist of associated heavy and light chains, which fall apart under reducing conditions.

## Conclusion

Traditional SDS-PAGE serves as a standard method for protein analysis, however, SDS-PAGE is very labor-intensive, time-consuming and difficult to standardize. In contrast the Agilent 2100 bioanalyzer provides a fast, standardized method with automated and detailed data analysis. Depending on the kit chosen, proteins from 5-200 kDa can be analyzed with a resolution of 10 %, or better, throughout the size  $range^{2,3}$ . These attributes of speed, automation and resolution make the Agilent 2100 bioanalyzer the perfect tool for analysis of bispecific antibodies.

## **References**

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Published June 1, 2003 Publication Number 5988-9651EN

