

The Application of the 2100 Bioanalyzer-Protein chip in the Biotechnology Industry

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Background

One of the requirements during the process of manufacturing development of a therapeutic protein is continuous monitoring of several key parameters namely, concentration, structural integrity and purity at selected stages of the product life cycle. Traditionally conventional methods such as HPLC, SDS PAGE electrophoresis and ELISA assays are being used to obtain this information. These methods are time consuming, labour intensive and individually offer only single parameter of those required. These techniques require multiple skilled operators and can result in protracted time lines and are usually located away from the fermentation sites.

At Lonza Biologics the 2100 Bioanalyzer-Protein 200 plus kit has been used to determine product purity, integrity profile, size and titre using minimum sample volume and time resource input. The 2100 Bioanalyzer is part of the new generation of miniaturised μ -TAS (Micro total analytical system) allowing the capture of multiple analytical parameters for product quality including the efficiency of antibody assembly in cells post the transfection process. This technology is essentially a miniaturised capillary electrophoretic system with microfluidics application confined to a chip.

The 2100 Bioanalyzer was used to monitor the quantity and quality of monoclonal antibodies (MABs) expressed using Lonza Biologics GS-Gene expression system. Suitable medium selection was facilitated using 2100 Bioanalyzer screening. In-process culture supernatant and purification samples from laboratory-scale and pilot scale fermentation processes were directly tested for product quality and quantity. The data generated using the 2100 Bioanalyzer were rapid and proved useful in the optimisation of the fermentation and purification processes.

This article outlines the data obtained for IgG4 tested at various stages of the manufacturing development process. We demonstrate that the 2100 Bioanalyzer will have a positive impact on the quality and efficiency of the production processes for therapeutic proteins.

Introduction

The number of therapeutic antibodies and recombinant proteins developed has risen dramatically over the past decade. This explosion in therapeutic antibody research was followed with major therapeutic successful products such as Rituxan® and Herceptin®, the first therapeutic antibodies accepted by the Food and Drug administration (FDA) for non-Hodgkin's lymphoma and breast cancer respectively

and more recently immunotherapeutics showed indications in the inhibition of prion replication and slowing down the prion disease (White *et al.*, 2003). Over 90% of the potential therapeutic antibodies are at the clinical trial stages. This trend is likely to increase with the timing of genomic and proteomic research. The type of diseases targeted include arthritis (Kevorrkov & Futlik, 2000), asthma (Nelson, 2003), HIV (Tsamis, 2003) and stroke (Wiessner, 2003). Clinical trials and in market therapeutic antibodies are produced in vast quantities depending on the market requirements and trial demands.

Analysis of these products is vital throughout the product life cycle from development to final release. The current advances in proteomic research and increased rate of therapeutic antibody development requires more efficient technologies and platforms with multi-analytical/parameter capabilities to deliver the data rapidly to facilitate the anticipated throughput. This is where the 2100 Bioanalyzer (Figure 1) proves its usefulness.

This technique has been evaluated at Lonza Biologics for the analysis of a monoclonal antibody (IgG4). This is referred to as Lab on a Chip technology and is based on microfluidics where sample preparation, fluid handling and biochemical analysis are carried out within the confines of a microchip. The chips comprise of micro-fabricated channels that are a few μm in dimension in glass that create an interconnected network of gel matrix reservoirs and pathways. This technology has fully automated analytical and data processing capability.

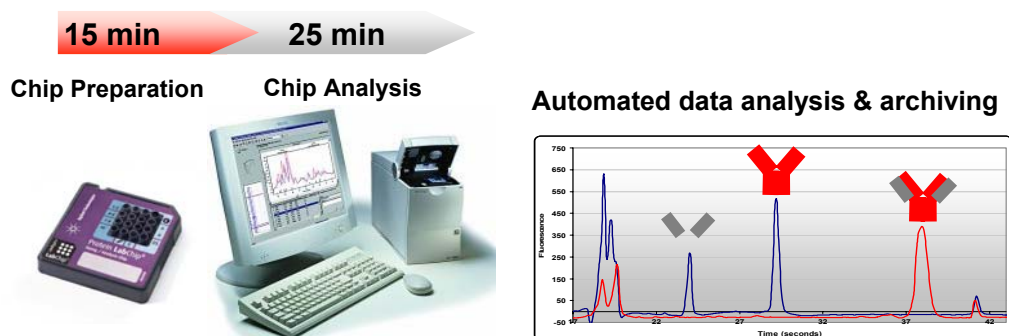
At Lonza Biologics we have employed the 2100 Bioanalyzer at selected stages of product manufacturing development. Some promising data were obtained.

Lonza is a Life Sciences driven company headquartered in Switzerland, with sales of CHF 2.54 billion in 2002 and operating 18 production and R&D facilities in 8 countries. It employs 6200 people worldwide and is the leading custom manufacturer of active chemical ingredients, intermediates and biotechnology solutions (using mammalian cell culture and microbial fermentation techniques) for the pharmaceutical and agrochemical industries. For more information on Lonza please visit the company's website at www.lonza.com.

Method and Sample Preparation

4 μl of sample was added to 2 μl of sample buffer containing SDS, two internal standards myosin and bradykinin ($\pm \beta\text{-MCE}$). Sample was heated in a boiling water for 1 minute. After cooling the sample was spun at 13k RPM for 15 seconds. This was followed by the addition of 84 μl of distilled water. One of the 16 wells of the protein chip was then filled with 12 μl gel-dye matrix and was pressurised for 60 seconds to allow it to enter into the micro-channels. The other wells were used for applying gel-dye matrix reservoir and destaining solution. 6 μl of samples and a ladder (molecular weight marker) was applied into the protein chip wells. Proteins are separated by the acrylamide sieving process of the gel and stained with a red fluorescent dye (RFD). The RFD interacts with the protein sodium dodecyl sulphate (SDS) micelles. The protein chip was loaded onto the 2100 Bioanalyzer. The 2100 Bioanalyzer contains 16 high voltage powered electrode pins, which touch the samples and gel-dye matrix in the wells thereby forming an electric circuit. This circuit allows the samples to be mobilised from the wells into the separation channels. Each sample is then sequentially separated and detected by the laser induced fluorescent (LIF) detection (670-700 nm) within 45 seconds. After the analyses is complete data are presented in the form of electropherograms (graphic plot of fluorescent units against time), tabular data format and gel-like image. All the reagents are provided in the 2100 Bioanalyzer-Protein 200 plus kit.

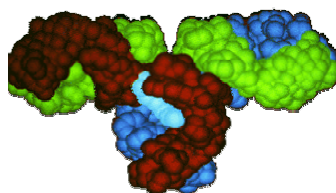
Figure 1 2100 Bioanalyzer-200 plus protein chip and typical electropherograms generated for MAb analysis under reduced ■ and non reduced ■ conditions.



The Multiple Applications of the 2100 Bioanalyzer in the Manufacturing Development Process of Therapeutic Antibody

**Antibody
assembly and
integrity after
initial cloning**

**Medium
selection for
optimal
antibody
production**



**Purification
development
process
support**

**Laboratory scale
fermentation
process-product
titre and quality
assessment**

Applications of 2100 Bioanalyzer

Antibody analysis using the 2100 Bioanalyzer at early stages of the manufacturing development process (Figure 2a & 2b) showed that titre and antibody integrity information are essential in the selection process of cell line clones. Conventionally ELISA is used to assess assembled antibody titre. The 2100 Bioanalyzer generates the titre data as well as additional sizing and antibody integrity information. Each cell line can be assessed for the amount of intact antibody produced and the level of unassembled heavy chain and light chain fragments can potentially be quantified and trended. This allows and facilitates rapid decision making process as to which cloned cell lines to progress further.

Rapid trending of the product titre and overall profile of the fermentation medium containing the product in real time *at line* was only realised using the 2100 Bioanalyzer and specific Lonza Biologics sample preparation method. The titre, % purity (intact antibody) and the amount of other product related fragments were trended *at line* (Figures 3, 4A & 4B). This application holds a very important prospect for the determination of product quality and quantity *at line*. At fermentation development stages the normal practice of trending product titre can be part of a product profile rather than a single titre value (Figure 4A). Figure 3 shows the intact antibody peak and other peaks which correspond to other antibody fragments,

including 144 kDa, 89 kDa, 56 kDa and 51 kDa. All these fragments can be trended in addition to that of intact antibody (Figure 4C). Titre and overall integrity of the intact antibody and fragments throughout the fermentation process allows the fermentation scientist to make a better and a more informed decision with regard to the product quality and fermentation process optimisation.

Product purification processes require on-going monitoring of the status of the antibody after each purification step for titre, % purity and quality of the product. 2100 Bioanalyzer provided these data *at line*. The data have direct impact on the purification process development strategy (Figure 5). Again the traditional techniques take long to generate the necessary data required to optimise purification processes. Figures 5A-5D show data generated after each step of a purification process. 5A being the product at the pre-purification step (culture supernatant load material). 5B shows the profile of the product after removal of the impurities after the first purification step. Figure 5C depicts the profile after a further purification step. Figure 5D shows the product profile after the final purification step. Table 1 shows the stepwise reduction of protein impurities in the cell culture supernatant.

The 2100 Bioanalyzer was used for the medium selection process. Various medium specifications, including protein free media were used to grow CHO cells and the intact antibody, light chain (LC), and heavy chain (HC) products were analysed (Figure 6). This application is an extension to the usual methods used to select appropriate media. Medium selection processes often only use cell viability and product titre. Using the 2100 Bioanalyzer allows the monitoring of the overall performance of the medium with regard to product quality and quantity.

Antibody Assembly Development Process

Figure 2a Electropherograms for the profile of culture supernatant containing intact monoclonal antibody and other fragments obtained from preliminary overgrown transfected cells. FL6 fluorescence response is presented on the Y₂ axis.

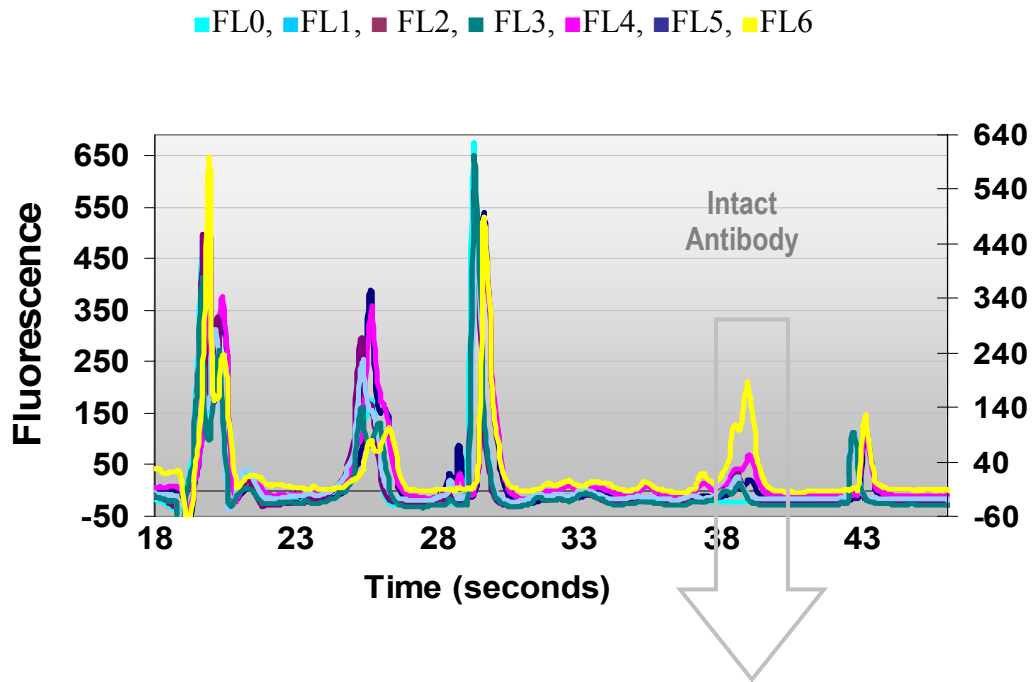
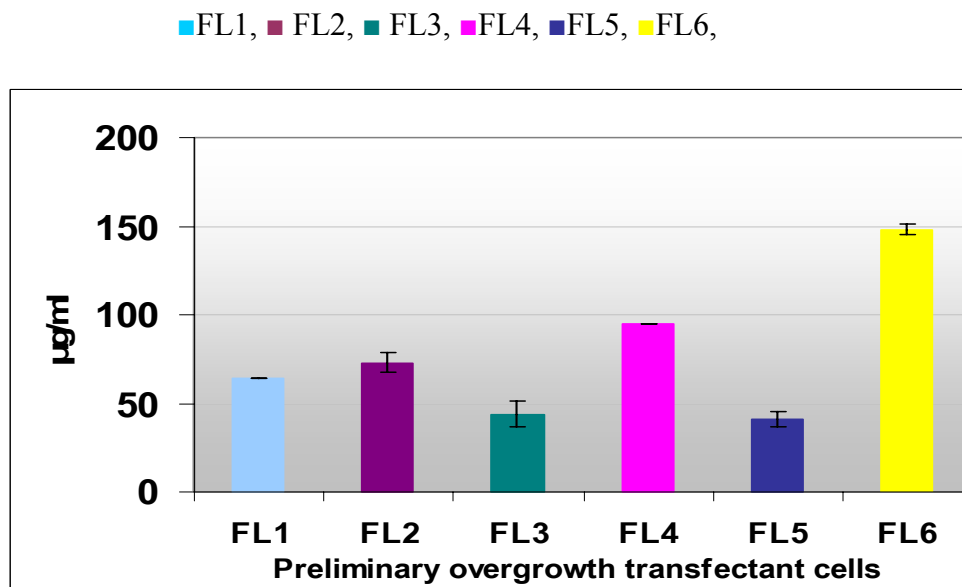


Figure 2b Intact monoclonal antibody titre



Fermentation Process Development

Figure 3 Electropherograms of intact monoclonal antibody and other fragments in culture supernatant analysed using 2100 Bioanalyzer *at line*. Samples were collected at different intervals across two weeks pilot scale fermentation process.

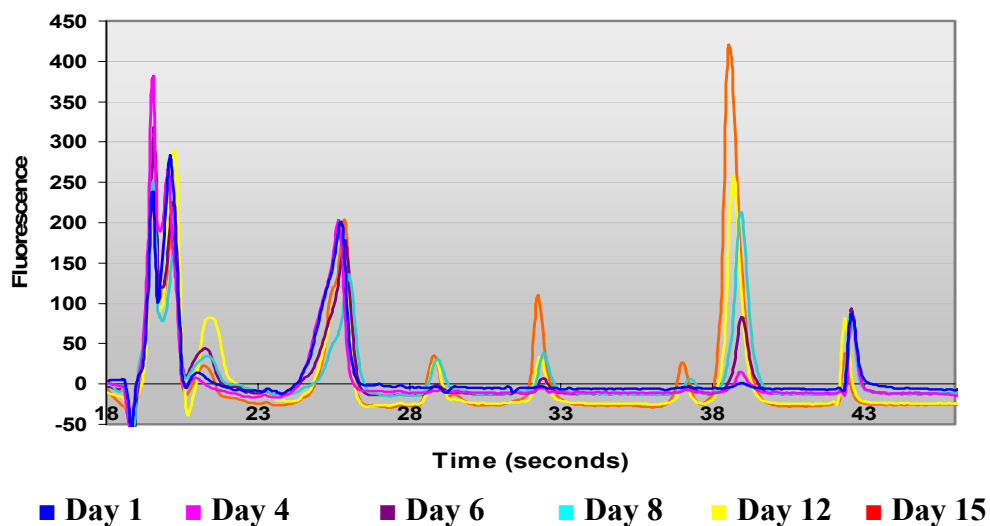
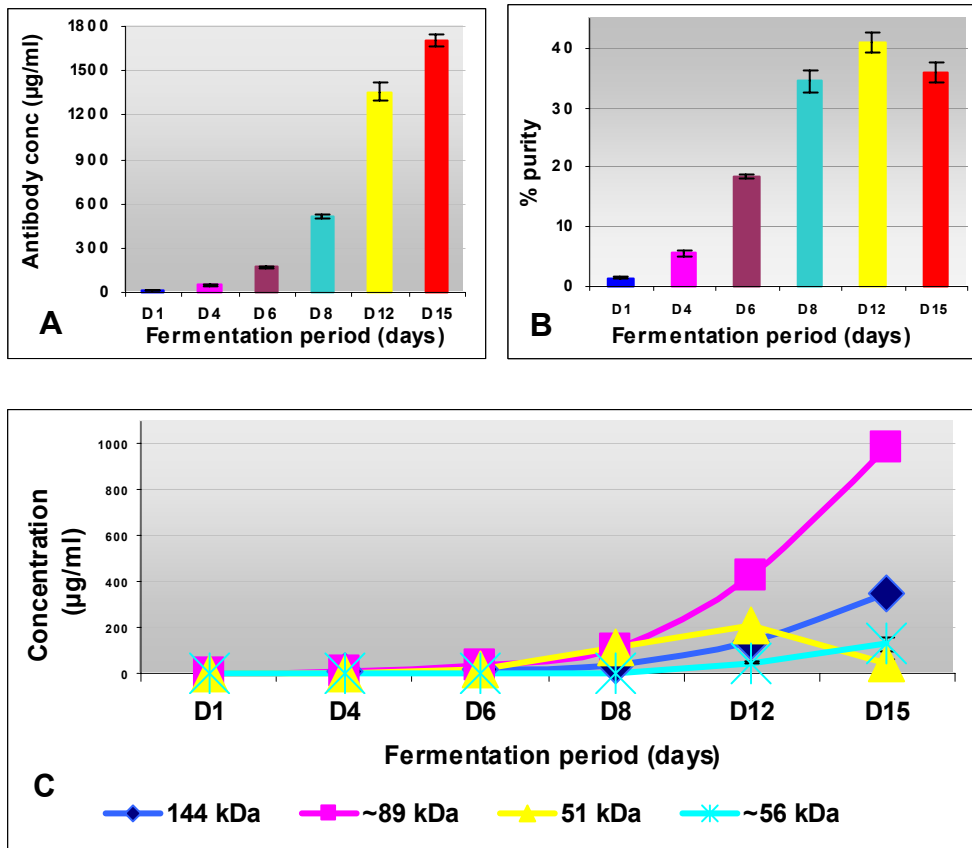
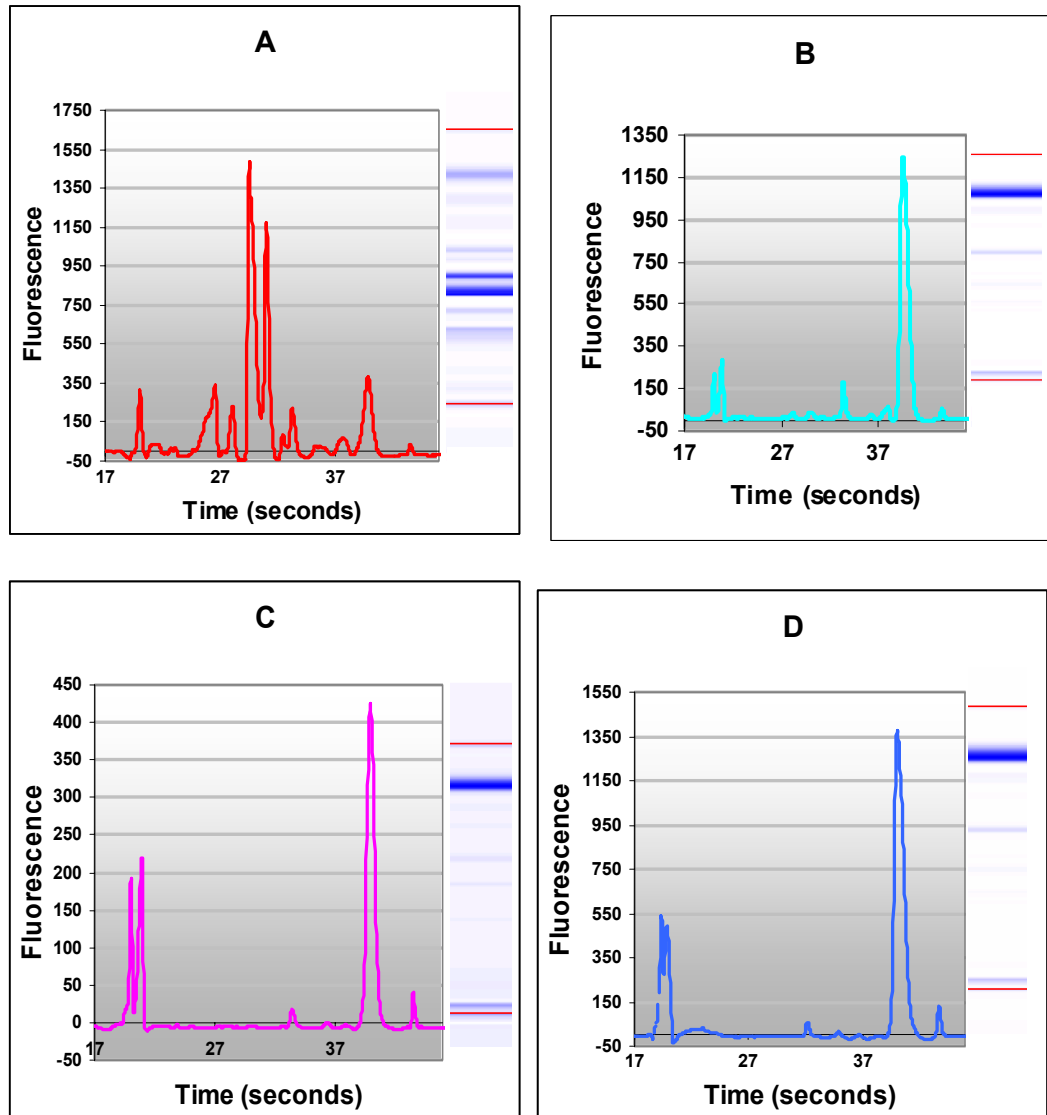


Figure 4 **A** The titre of the intact antibody in cell culture supernatant analysed *at-line*.
 B The % purity (intact monoclonal antibody).
 C The titre of antibody fragments across fermentation process.



Purification Development Process

Figure 5 Monoclonal antibodies undergo several stages of purification. At each stage of purification process development, the 2100 Bioanalyzer was used to assess product quality and purity.



A ■ Unpurified load
C ■ Post-purification process 2

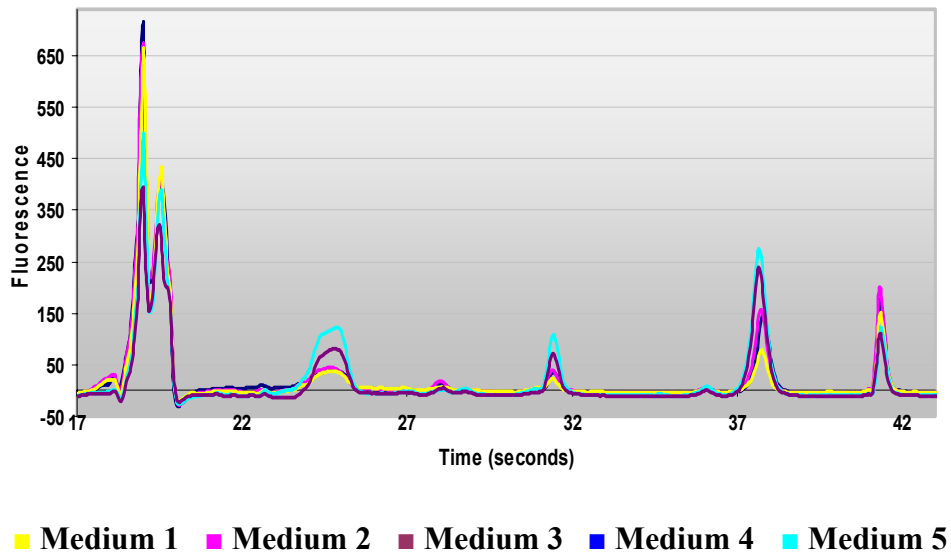
B ■ Post-purification process 1
D ■ Post-purification process 3

Table 1 Purification and separation process of the intact antibody, antibody fragments and other impurities, P1 to P3 are the different purification steps.

Size (kDa)	Load	P1	P2	P3	Final purified product
14	√	√			
15	√	√			
23					
33	√				
38	√	√			
43	√		√		
51	√	√			
56			√		
64	√	√			
80	√				
141	√		√		
114	√				
118	√				
123			√	√	
91	√		√	√	√ (~1%)
167	√	√	√	√	√ (~99%)

Medium Selection Process

Figure 6 Monoclonal antibody grown in various media specifications. Product profile is shown in the following electropherograms. Product concentration in medium five was higher than the other media.



Concluding Remarks

The data presented here show the applicability of the 2100 Bioanalyzer for the simultaneous determination of the titre, integrity and % purity of monoclonal antibodies throughout the manufacturing and purification development process.

2100 Bioanalyzer can be used for the analysis of both monoclonal antibodies and other recombinant proteins produced by mammalian and microbial cell culture.

The 2100 Bioanalyzer allows *at line* analysis at a rapid pace relative to other conventional techniques that is complete analyses and data generation of up to ten samples in 40 minutes (including data manipulation time). This introduces improved efficiency in the process of therapeutic antibody manufacturing development.

This ultimately reduces resources spent during the development of biopharmaceuticals and the time line required to manufacture products for clinical trials and market supply.

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