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WHITE PAPER

Robust Recombinant Antibody Engineering:
Equivalency Studies Of Biosimilar Monoclonal Antibodies

Summary

Bio-Techne leverages the decades of experience of R&D Systems' antibody manufacturing to support both research and diagnostic communities with high-quality, reliable antibodies. Bio-Techne's expertise spans the spectrum from *de novo* antibody engineering to recombinant antibody conversion. We've taken this engineering know-how and deployed it to bring you a range of biosimilar monoclonal antibodies for research use. Bio-Techne's biosimilar antibodies are manufactured from publicly available sequences, with no alteration or truncation. In this whitepaper, we demonstrate that Bio-Techne's biosimilar antibodies, intended for *ex vivo* research use only, are equivalent to the original therapeutic antibodies in functionality and kinetics, as well as surface charge.

Introduction

Biologics are becoming increasingly common in the treatment of various diseases including cancer and inflammatory and autoimmune diseases. Biosimilars are biologics that demonstrate therapeutic equivalency to the original approved drug. The Food and Drug Administration (FDA) defines a biosimilar as a biologic with "no clinically meaningful differences in terms of safety, purity, and potency (safety and effectiveness) from an existing FDA-approved biologic". [1] Due to the molecular complexity of biologics, biosimilars are not required to be identical to the original approved drug, but only comparable or equivalent to the reference therapeutic drug in terms of purity, functionality, pharmacokinetics, immunogenicity, and safety.[2] As of December 2023, the FDA has approved 45 biosimilars within four product categories: (1) Tumor necrosis factor-alpha (TNF-α), (2) insulin, (3) monoclonal antibodies, and (4) granulocyte colony-stimulating factor.[3]

For research use, biosimilar monoclonal antibodies are commonly used as cost-effective alternatives for studying and characterizing specific disease targets. Biosimilar antibodies can serve as critical controls to benchmark novel treatments.

Through The Pipeline: From Sequence To Antibody

Biosimilar antibodies are developed and manufactured with Bio-Techne's recombinant antibody engineering and expression platform. This robust and versatile platform is used to express hundreds of recombinant antibodies from many species including mouse, rat, rabbit, llama, and goat. Bio-Techne has a diverse array of antibody engineering capabilities, including Fc species and isotype swaps, multivalent antibodies, camelid VHH, F(ab) fragments, single-chain variable fragments (scFvs), immunocytokines, chimeric antigen receptors (CARs), and recombinant conversion of hybridoma cell lines. Many *de novo* recombinant antibodies are derived from antigen-specific B cells from immunized animals. Our proprietary immunogen design tools and immunization protocols, coupled with a modernized multi-species recombinant antibody process, has generated high affinity antibodies against challenging targets, such as small molecules, phosphorylated peptides, and complex integral membrane proteins like G-protein couple receptors (GPCRs) and ion channels.

Bio-Techne's biosimilar pipeline is shown in Figure 1. Briefly, antibody sequences are obtained from public databases and engineered *in silico* to develop stable production vectors that yield high level antibody expression without modification to the

drug. Engineered antibody light and heavy chains are synthesized to an optimized expression vector for small-scale expression in our proprietary HEK293 platform. Our high throughput transfection process allows for rapid and concurrent interrogation of large panels of recombinant antibodies to identify the ideal antibody that is suitable for the intended functionality and application. Antibodies are manufactured under controlled conditions and undergo rigorous quality control testing to ensure lot-to-lot consistency and performance.

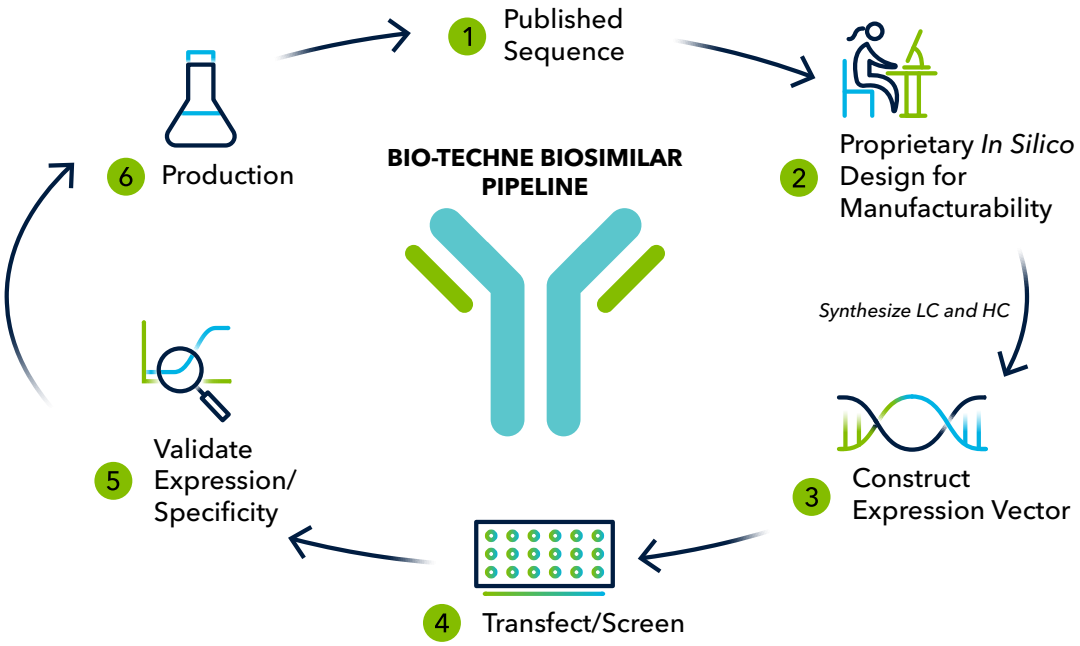

Figure 1. Biosimilars are developed and manufactured with Bio-Techne's recombinant antibody engineering and expression platform. Briefly, published biosimilar sequences are engineered for optimal expression and stability without protein alteration, synthesized to our proprietary expression vector and screened in our high-throughput HEK 293 system. Manufacturable clones with validated specificity are produced on our production platform that provides high antibody yields with high lot-to-lot consistency.

Equivalency Studies With Reference Therapeutics: The Most Similar Biosimilars

In this whitepaper, we show that biosimilar antibodies manufactured by Bio-Techne are equivalent to the reference therapeutic product by ELISA binding, capillary isoelectric focusing (cIEF), surface plasmon resonance (SPR), and a bridging assay with anti-idiotype antibody.

Materials And Methods

Direct ELISA: Purified recombinant protein was used to confirm binding equivalency of the biosimilar to the reference therapeutic antibody. The protein was diluted in 1x TBS to a concentration of 400 ng/mL. A 96-well microtiter plate was coated with 100 mL of the diluted recombinant protein, followed by blocking with 10% BSA solution for 1 hour. The plates were then washed 2x with wash buffer. Ten 3-fold serial dilutions of the biosimilar and reference material were introduced into each protein-coated well followed by incubation with goat anti-human IgG-HRP detection antibody. The plates were then washed 2x with wash buffer followed by colorimetric detection at 450 nM.

Surface charge characterization using capillary isoelectric focusing (cIEF): Maurice (Catalog # 090-000, ProteinSimple a Bio-Techne Brand) is a cutting-edge benchtop platform for both capillary electrophoresis SDS (CE-SDS) and rapid cIEF analyses. Using the cIEF capability, the user can interrogate up to 100 samples in a single batch run (Catalog # PS-MDK01-C). The system uses cartridge-integrated fluidics (Catalog # PS-MC02-C) to facilitate a highly simplified workflow with minimal user intervention. A 1.25x cIEF master mix was made containing 0.4375% methylcellulose, 2.75% Pharamlyte® (GE Healthcare), pH 8 to 10.5, 4 mM L-arginine, 20 mM HCl, and a 1:80

dilution of reconstituted Maurice cIEF pI 8.40 and 9.99 markers and vortexed to mix. Biosimilar and reference material samples were prepared by diluting the 10 mg/mL stock with deionized water to either 0.5 or 1.5 mg/mL. 160 µL of 1.25x cIEF master mix was added to 40 µL of antibody sample, vortexed, and centrifuged at 13,000g for 3 minutes. The top 160 µL of centrifuged solution was transferred to a sample vial (Catalog # 046-017, 046-138) or plate well (Catalog # 046-021), centrifuged, and placed in the Maurice. Samples were focused at 1000 volts for 1 minute, followed by 3000 volts for 7 minutes.

Biosimilar binding analysis by surface plasmon resonance (SPR): Surface plasmon resonance equivalency experiments were performed with the Carterra LSA® instrument. The immobilization of the biosimilar antibodies was performed via capture by Protein A/G (Bio-Techne/PrimeGene). To prepare the capture surface, 100 ug/ml Protein A/G in 10 mM NaOAc, pH.4.5 buffer was coupled to an HC30M chip (Carterra) by amine reactive NHS/EDC covalent chemistry followed by blocking the unreacted areas of the chip surface with 1 M ethanolamine. Reference cells were prepared similarly. Following preparation of the Protein A/G surface, the biosimilar and reference antibodies were immobilized by printing at a concentration of 0.2 ug/ml prepared in SPR running buffer HBS-EP (10 mM HEPES, pH 7.4, 150 mM NaCl, 0.05% Surfactant P20, 3 mM EDTA, and 0.5 mg/ml BSA). Binding responses of each indicated target analyte to their corresponding biosimilar antibody were measured through serial injection of the analyte proteins across the biosimilar antibody capture surface. Analytes were serially injected at increasing concentrations of 0.8, 2.5, 7.5, 22, 67, and 200 nM in HBS-EP buffer to yield single cycle kinetic binding response curves. The experimental data was double referenced by removing the trace background binding responses measured in the absence of either ligand or analyte. The reference subtracted experimental data was fitted using a 1:1 ligand-analyte Langmuir kinetics binding model. The values for the best-fit kinetic rate constants of association (k_{on}) and dissociation (k_{off}), along with the equilibrium dissociation constant (KD = k_{on}/k_{off}) are reported.

Results

Comparability of research-grade biosimilars to their therapeutic reference material is a requirement for customers seeking to benchmark new treatments or design functional assays against a therapeutic target. Customers seeking to benchmark therapeutic advancements need a reliable source of biosimilars to accelerate their research programs. Unlike small molecules, antibodies are large biologics with significant structural complexity requiring a development and manufacturing process that can produce highly similar versions of the original innovator therapeutic. Using our proprietary recombinant antibody engineering and expression platform, we have been producing biosimilars for several years. Here we provide evidence that our biosimilars are equivalent in: (1) target recognition and binding (ELISA), (2) charge variant profiles (Maurice-enabled capillary isoelectric focusing or cIEF), and (3) binding kinetics and epitope specificities (SPR).

Direct ELISA Binding Studies

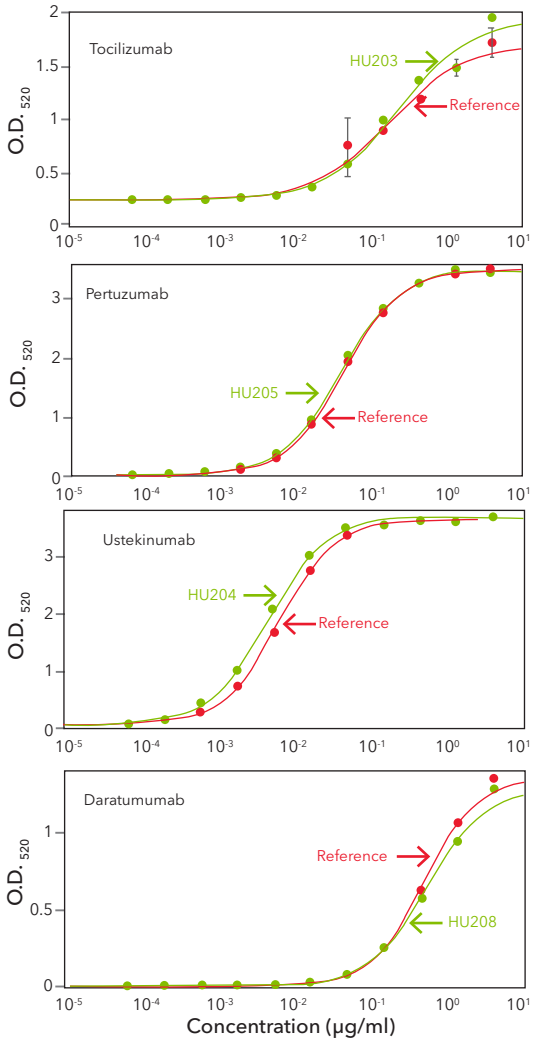

Figure 2. Direct ELISA curves demonstrating equivalency to the reference therapeutic. The target protein was coated onto the microplate well surface, followed by binding of the antibody. A goat anti-human IgG-HRP conjugate was used for detection.

The binding equivalency of Bio-Techne's biosimilars to the reference therapeutic is depicted in Figure 2. The direct ELISA in this context serves a two-fold purpose: (a) as an identity test to show binding to the intended target, and (b) as a demonstration of equivalency. The binding curves shown in Figure 2 establish the equivalency to the reference therapeutic in sensitivity and over a broad dynamic range of dilutions.

Capillary Isoelectric Focusing (cIEF) Profiles

Charge variant analysis using the Maurice capillary electric focusing (cIEF) platform demonstrates nearly identical profiles between the biosimilars and therapeutic antibodies (Figure 3).

cIEF is used as a standard practice in the therapeutics industry to look for manufacturing consistency as well as proof of identity prior to release of each lot of protein. Monitoring of surface charge characteristics is a critical step in quality control of therapeutics. cIEF is therefore an indispensable tool for interrogating the charge profiles of antibodies and is an indicator of lot-to-lot reproducibility, as well as comparability between a biosimilar and its therapeutic counterpart. The results shown in Figure 3 demonstrate that Bio-Techne's recombinant antibody platform utilizing an engineered HEK293 cell line preserves the humanized post-translational modifications of the original therapeutic.

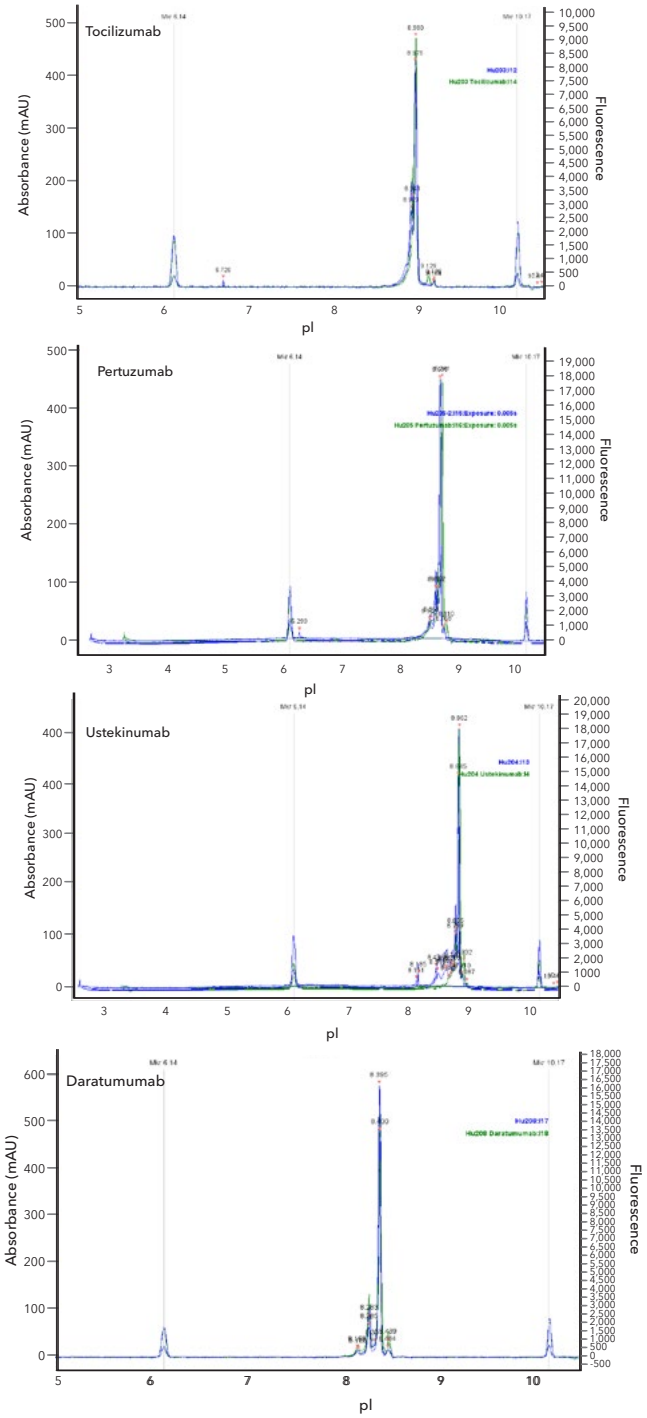

Figure 3. Isoelectric focusing (cIEF) equivalency profiles of biosimilars compared to their therapeutic reference antibodies. The high throughput Maurice Ice cIEF platform was used to interrogate the surface charge characteristics of biosimilars and reference material. The Maurice Ice offers the capability to analyze up to 100 samples simultaneously in less than an hour.

Binding Kinetics Using Surface Plasmon Resonance (SPR)

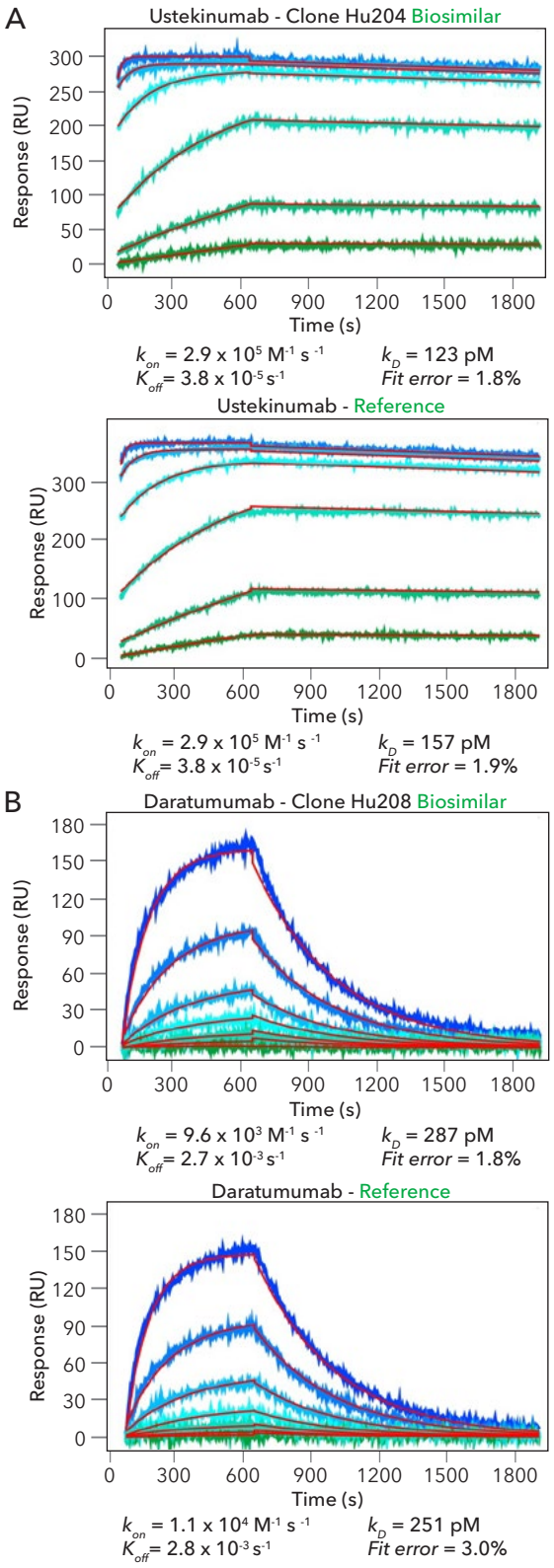

Figure 4. Surface plasmon resonance (SPR) analysis of binding affinities and kinetics of Ustekinumab and Daratmumab display equivalency between the biosimilar and reference therapeutic material. The Carterra LSA platform was used for SPR studies.

Surface plasmon resonance (SPR) is broadly used for the study of antigen-antibody kinetics and affinities. Typically, SPR measurements do not involve any labeling, and are based upon the quantitative binding of the analyte to its ligand that is attached to a microfluidic chip. For this study we compared the binding of the biosimilars and the reference antibodies to Protein A that was immobilized onto the chip. The results, shown in Figure 4, demonstrate that the binding kinetics of the biosimilars are equivalent to the therapeutic reference antibody. Using SPR, we show that the association rate constant, k_{on}, and dissociation rate constant, k_{off}, values of both the biosimilar and reference antibody are almost identical.

Taken together, biosimilar antibodies produced by Bio-Techne clearly demonstrate equivalency in the three assays described above. An overall view of equivalency is depicted in Figure 5 on the next page pointing to the robust and versatile nature of our recombinant antibody platform.

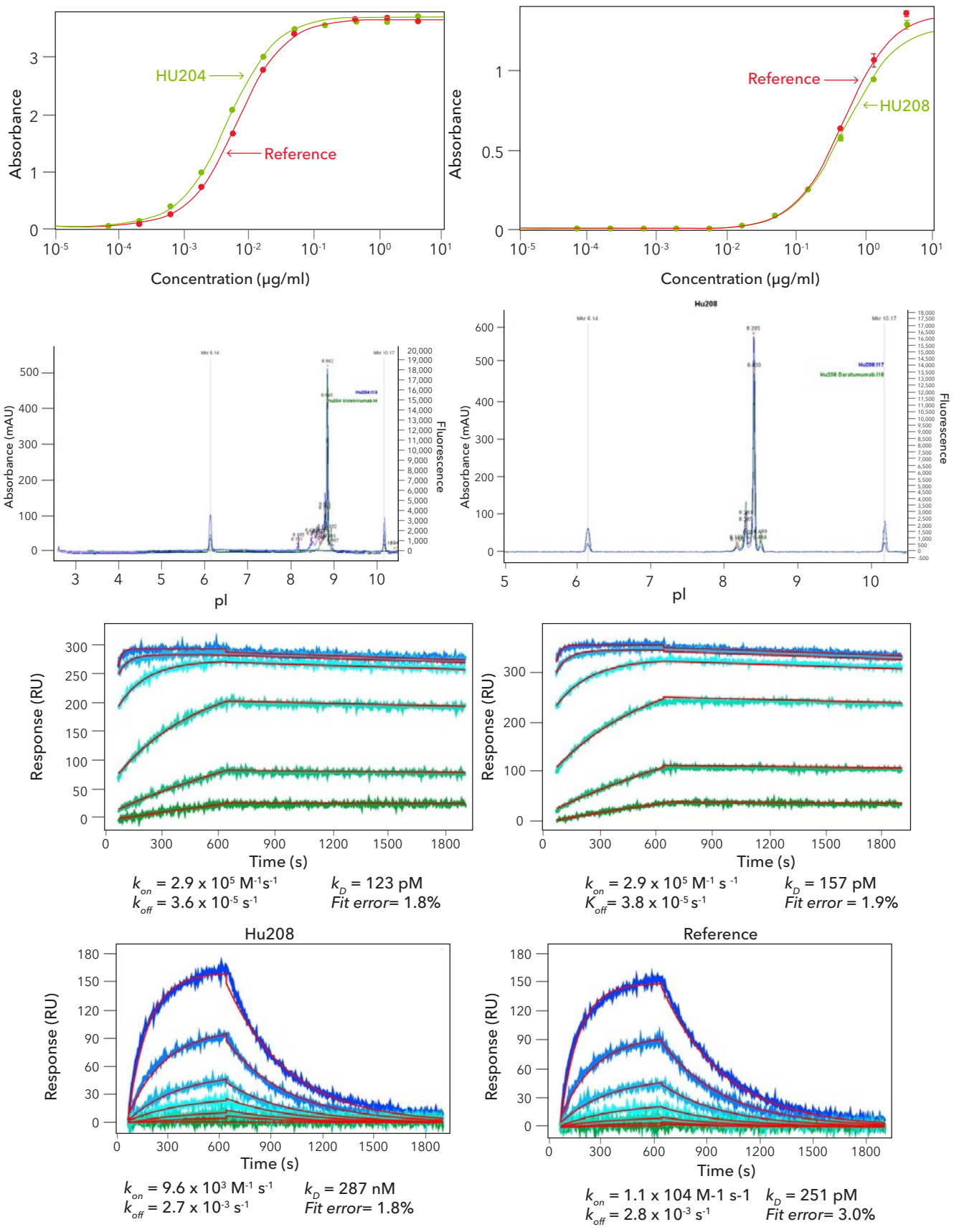

Figure 5. A comprehensive analysis of Ustekinumab and Daratumumab biosimilars and reference and material show strong equivalency.

Bridging Assay Using Adalimumab Anti-idiotypic Antibody

In addition to the assays described above, we also investigated if an anti-idiotype antibody was generated using a biosimilar bound to the therapeutic reference. Using the Anti-Adalimumab anti-idiotype antibody (Catalog # MAB9616), we demonstrate that recognition of both the biosimilar and the therapeutic antibody by our anti-idiotype is equivalent (Figure 6).

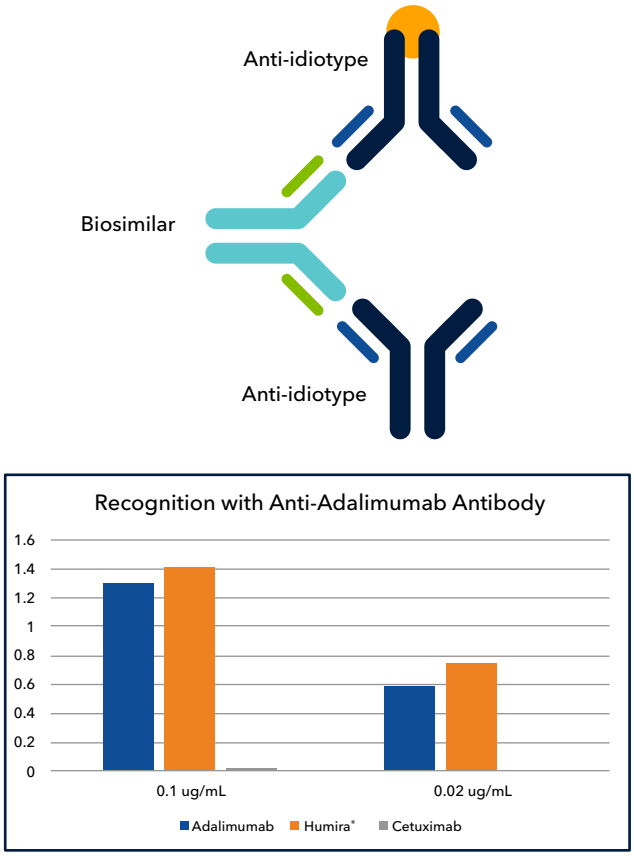

Figure 6. Bio-Techne Adalimumab performs similar to Humira® in bridging assay with anti-idiotype antibody. (A) Schematic representation of bridging assay with biosimilar antibody and anti-idiotype antibody. (B) Results of bridging assay for Adalimumab Biosimilar (blue), Humira® reference product (orange), and Cetuximab (grey) with Anti-Adalimumab antibody.

Conclusions

Biosimilars offered by Bio-Techne are equivalent to their reference therapeutic antibodies, making them suitable for use in bridging assays and as benchmarks in novel therapy programs. Equivalency was confirmed in multiple assays including, direct ELISA binding, cEIF on Maurice, SPR, and anti-idiotype bridging assays. These studies demonstrate the robustness of Bio-Techne's recombinant antibody engineering service to provide the research and diagnostic communities with high quality biosimilars for research and development.

References

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