APPLICATION NOTE

Recombinant Antibody Cloning and Engineering Platform to Ensure Performance and Consistency for Long-Term Studies

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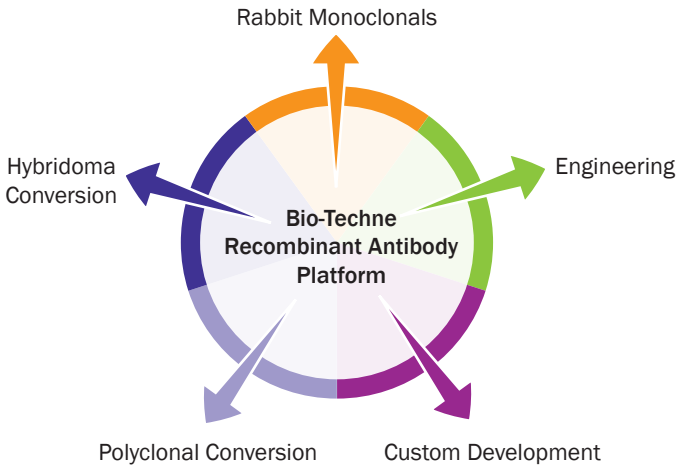
INTRODUCTION

Obtaining consistent immunoassay performance over the course of time has long been an area of concern for researchers. Challenges with drift in affinity and expression levels of both monoclonal and polyclonal antibodies can contribute to assay variability, and compromised results. Recombinant antibodies offer several advantages over traditional antibodies as research reagents. The expression of recombinant antibodies through an expression vector system eliminates loss of affinity due to genetic drift, and ensures an antibody product with minimal differences in lot to lot performance and yield. Another advantage of recombinant antibodies is the capability to engineer and tailor antibodies for specific applications, as well as enhance expression levels.

In order to ensure the continuous and dependable supply of high quality antibodies for consistent performance, a robust recombinant antibody platform has been designed to clone and express antibodies from an array of species and sources including hybridoma and peripheral B-cells. The platform can be used to generate antibodies in several formats including, Fab, Fab'2, scFv, bi-specifics, tagged, engineered Fc, isotype and species swap, and humanized versions. This platform guarantees yields that provide the gram quantities required for the development of diagnostic, therapeutic reagents and research reagents. The platform can also be used to convert polyclonal antibodies to recombinant monoclonals, eliminating the need to immunize a new group of animals every few years. *Conversion to a recombinant monoclonal effectively renders the antibody immortal and future supply is guaranteed.* The cloning and expression platform is used to produce recombinant antibodies encompassing several applications, including Western Blot, several formats of ELISA, flow cytometry, immunohistochemistry, as well as diagnostics platforms. As a demonstration of the platform's capabilities to produce high quality antibodies with consistency between lots, we present data demonstrating that recombinant monoclonal antibodies converted from either the polyclonal or hybridoma system are bioequivalent to the original antibody.



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METHODS

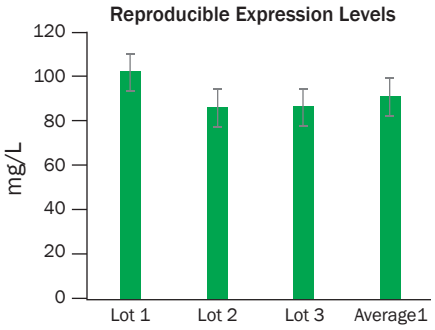
cDNA for heavy (HC) and light chains (LC) was amplified from (1) hybridoma cells for conversion into recombinant monoclonal antibodies; and (2) rabbit and goat blood B-cells for the conversion of polyclonal to recombinant monoclonal antibodies. For conversion of hybridoma monoclonal to the recombinant form, variable regions of the light and heavy chains were first sequenced to confirm that the cell population was clonal (1 HC and 1 LC). Conversion of rabbit and goat polyclonal antibodies to the recombinant version involved the isolation of B-cells expressing the antibody of interest, culture of the cells in a proprietary medium for about 7 days followed by cDNA amplification of the HC and LC variable regions. Several cDNA clones from the B-cell population were sequenced to identify 2–4 dominant heavy and light chains. For both hybridoma and monoclonal conversion, the HC and LC variable regions were cloned into optimized expression vectors. Following confirmation of activity, expression was scaled up in 1.0 and 3.0-L bioreactor cultures. Three independent lots of each antibody were grown to confirm consistency between lots.

Gram scale expression level per bioreactor culture.

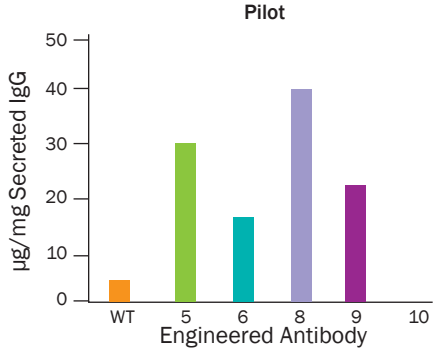
Traditional hybridoma methods require 5x to 10x greater media volume and 2x more time to produce equivalent amounts.

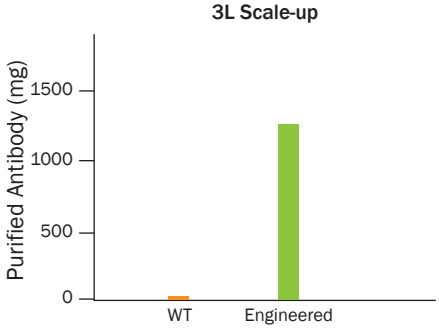
Developing a Production System

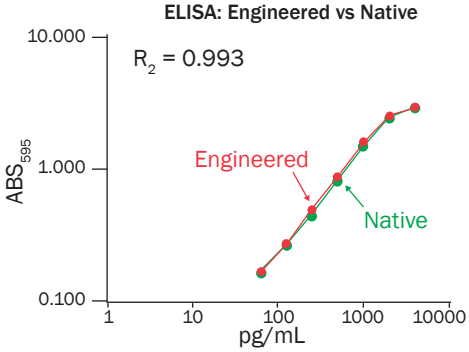
- Expression vector was designed and optimized for maximum yield
- Mammalian cell transfection SOPs were scaled up
- Unique cell culture medium was developed
- In-house engineered bioreactors were utilized



Direct Engineering Enhances Antibody Expression





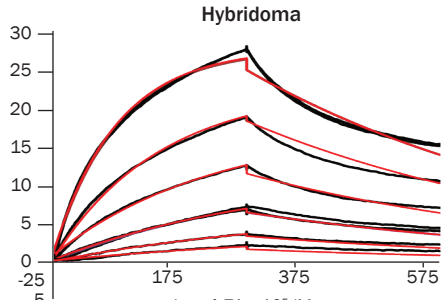


SUMMARY

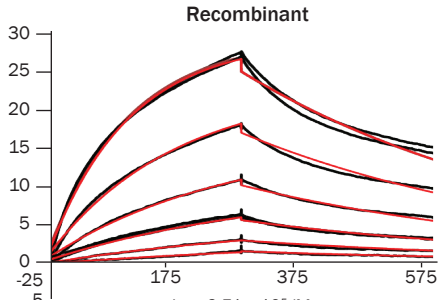
Recombinant antibodies from hybridoma clones were bioequivalent to the original antibody for all applications. High expression levels, ranging from 0.5 g to greater than 1.5 g, of antibodies were derived from either hybridoma conversions or B-cell clones. Typically, expression levels of recombinant antibodies derived from hybridoma clones were significantly greater than antibodies from the originating fusion clone. No statistical variation in either activity or expression levels was observed between independent lots. The full-length antibodies were also engineered into various formats depending upon the application.

The recombinant conversion process also eliminated several stability issues associated with hybridoma cell cultures. The conversion of either hybridoma or polyclonal antibody to the recombinant monoclonal version produce antibodies that are bioequivalent to the original source.

Biacore SPR Studies Demonstrating the Equivalency of Recombinant to Hybridoma Antibodies



k_a = 4.71 x 10^5/M · s
k_d = 2.05 x 10^{-3}/s
k_D = 4.35 pM



k_a = 6.51 x 10^5/M · s
k_d = 1.92 x 10^{-3}/s
k_D = 2.95 pM

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