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WHITE PAPER

Obtaining the Optimal Clone: Exploiting metabolic selection in mammalian cells for clonal enrichment, particularly for difficult-to-express proteins.

In 2015, the global revenue for protein therapeutics reached $174.7 billion[1], approximately two-thirds of which originated from protein therapeutics that were generated in mammalian cells[2]. Chinese hamster ovary (CHO) cells constitute the most significant workhorse in mammalian cell protein production. CHO cells are extremely versatile and easy to work with, and for many of the monoclonal antibody (MAb) therapeutics that are developed using CHO cells, it is often possible to achieve production levels of 2–5 g/L.

The life sciences industry is increasingly addressing life-threatening diseases with sophisticated and complex protein therapeutics, such as novel scaffolds, fusion proteins, bi-specific proteins, and cytotoxic cytokines and interferons. These next-generation therapeutics, which are often difficult-to-express proteins, have been accompanied by a new set of challenges with regard to their manufacturing. Generating manufacturing cell lines that secrete high levels of intact recombinant therapeutic proteins requires more than effective and stable integration of the recombinant gene into the manufacturing cell's genome. The cell clone must accommodate the transcriptional, translational, and secretion-related requirements of the recombinant protein while maintaining clonal stability and viability. As a result, much of the success of protein manufacturing depends on selecting the best clone.

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As these newer, more complex drug development candidates stress the secretory and metabolic machinery of manufacturing cell lines further, clonal selection becomes even more challenging. Consequently, there are fewer clones that meet all of the cellular and metabolic requirements and still produce high amounts of therapeutic protein, resulting in a larger "haystack" (cell populations) from which the "needle" (high-expressing clones) must be identified.

Recent studies have begun to determine the link between cell metabolism, growth, and recombinant protein expression. Vitamins are essential micronutrients for functional cell metabolism, growth, and propagation. Exploiting vitamins and metabolic selection in mammalian cells more effectively is a new approach for clonal enrichment. Whereas selection methods that are based on dehydrofolate reductase (DHFR) and glutamine synthase (GS)—which are required for the synthesis of metabolic intermediates, such as nucleotides and amino acids—have been successful in driving gene amplification and cell selection for manufacturing cell lines, they suffer from long selection times and yield polyclonal populations that express low to moderate amounts of recombinant protein. Selexis SA has developed a new addition to the SURE*technology* Platform™ called SURE*select*™, based on the stringent dependence of CHO cells on vitamin B5, which is a significantly faster and more powerful metabolic selection technology than these older methods.

CHO cells have a strict requirement for vitamin B5. CHO cells that are placed in media with 1/1000 the normal levels of vitamin B5 undergo irreversible changes in cell growth and division, dying within 6 days. Sodium-dependent multivitamin transporter (SMVT) is the protein that is primarily responsible for the uptake of vitamin B5 by CHO cells. Selexis scientists developed vectors, transfection methods, and culture protocols with which they co-expressed SMVT in CHO cell lines with various genes of interest. Even with a 1000-fold reduction in vitamin B5 levels in the media, they still obtained viable cells that expressed high levels of recombinant protein. Notably, they also demonstrated that the background level of poorly expressing clones in the cellular populations was low, making it much easier to isolate high-expressing clones. Further, when this new selection method was used in tandem with traditional antibiotic selection, the protein production levels were even higher, and high-expressing monoclonal and polyclonal cell populations that synthesized recombinant proteins were significantly enriched.

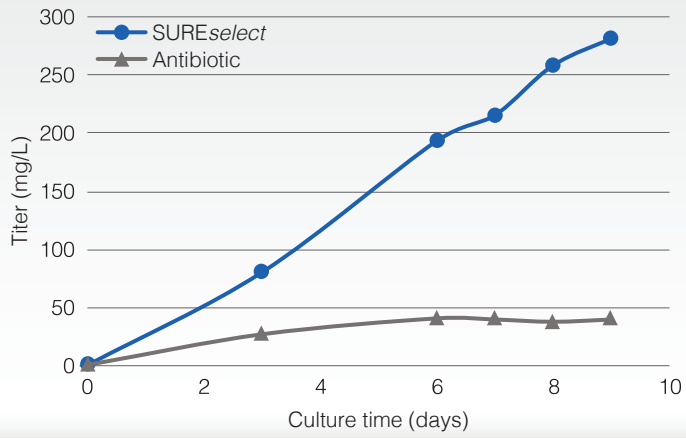
SURE*select* increases the production of difficult-to-express proteins. For example, interferon-beta is cytotoxic to CHO cells at high levels. Using SURE*select*

[1] BCC Research, "Global Markets and Manufacturing Technologies for Protein Drugs"
[2] MAbs (2015) 7(1):9–14.

in combination with antibiotic selection, it is possible to generate CHO cell pools that express IFN-beta at levels that are 5 times higher than what is typically achieved with antibiotic selection alone (from <50 mg/L to >250 mg/L in shake flask; see figure 1). SURE*select* has yielded cell lines with 4-fold greater production of difficult-to-express Fc-fusion proteins compared with antibiotic selection alone (from ~0.2 g/L to ~0.8 g/L in shake flask; see figure 2).

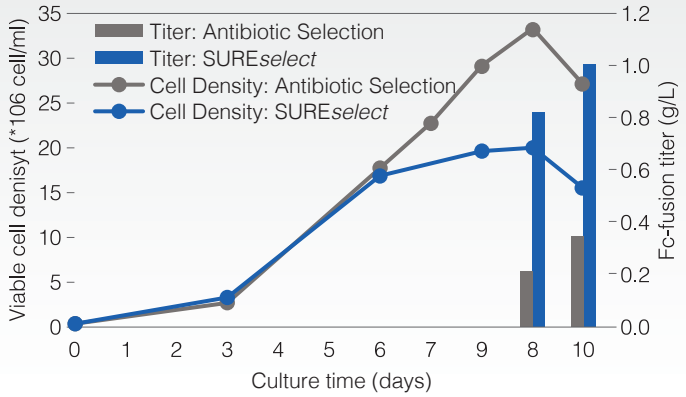
The selection of high-producing and stable mammalian cell lines is a potential bottleneck in the manufacture of biopharmaceuticals, particularly for difficult-to-express and cytotoxic proteins. The SURE*select* cell line selection method exploits the dependence of CHO cells on vitamin B5 for energy production, rendering it a powerful new tool for developing production cell lines. SURE*select* strengthens the commonly used antibiotic selection approach by increasing the signal-to-noise ratio of high-expressing clones over low-expressing clones. Although it is applicable to any therapeutic development program, the SURE*select* platform is particularly beneficial for programs that struggle with expression-related challenges.

FIGURE 1. PERFORMANCE OF CLONES PRODUCING INTERFERON BETA



Interferon-beta (IFN-ß) is cytotoxic to CHO cells at high levels and using antibiotic selection methods, the productivity of the isolated CHO cell clones is typically less than 50 mg/L. With SUREselect CHO cell pools expressed IFN-ß at levels 5 times higher (>250 mg/L in shake flask) than what is typically achieved with antibiotic selection alone. IFN-ß is a cytokine and is effective at much lower doses than monoclonal antibodies; hence, a production clone producing at greater than 250 mg/L is an economically viable solution.

Figure 2. Fc-FUSION



Comparison over time of cell density and titers from cells selected with either antibiotic selection alone or SUREselect. While the cell density using the SUREselect method is lower, the titer is actually higher indicating much higher producing clones.

KEYWORDS: Chinese hamster ovary, CHO, protein therapeutic, mammalian cells, difficult-to-express proteins, clone selection, antibiotic selection, vitamin B5, cell metabolism