

# A Simplified Method for Improving Antibody Product Quality and Function

## BalanCD Gal Supplement

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### INTRODUCTION

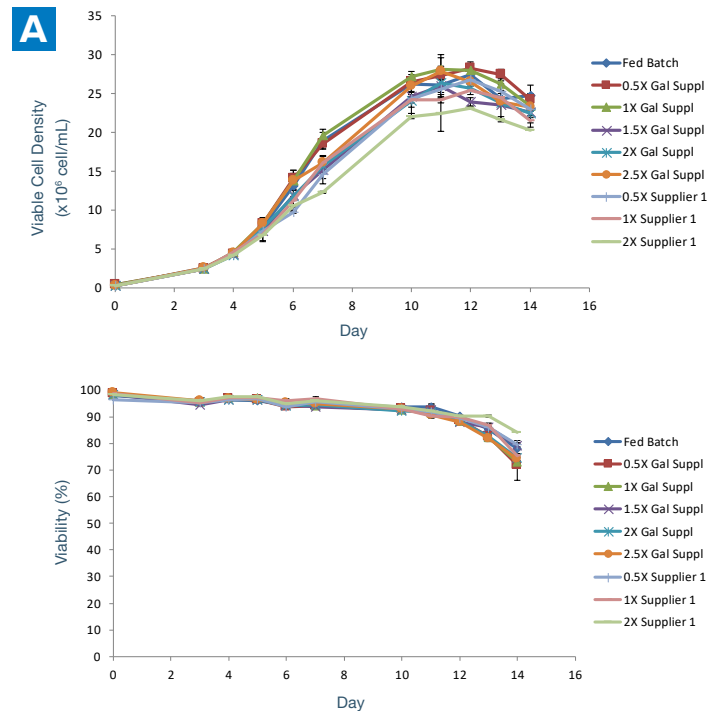
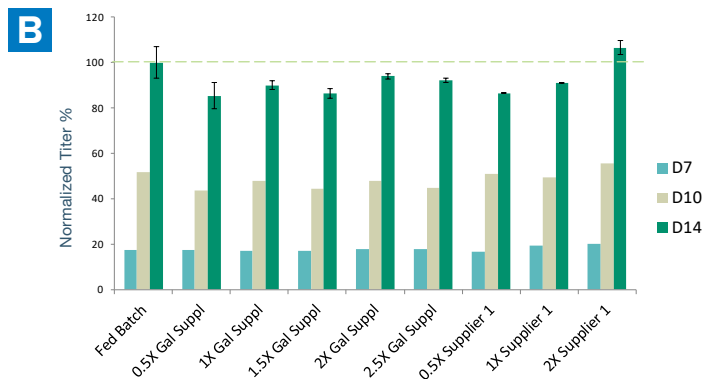
A critical aspect of product quality is glycosylation, or the attachment of sugars to organic molecules, which can significantly alter antibody (Ab) binding, function, half-life, and therapeutic effect.<sup>1</sup> For this reason, regulatory organizations closely monitor product quality and require therapeutic Ab manufacturers to control for product glycosylation. In an effort to mitigate the difficulty of controlling for glycosylation, FUJIFILM Irvine Scientific has introduced the BalanCD Gal Supplement, which aids in producing desired glycoprofiles by enhancing the galactosylation of proteins with a systemized, efficient feeding schedule.

Galactosylation, meaning glycosylation utilizing galactose, is of key interest due to its ability to influence product quality. To develop the BalanCD Gal Supplement, FUJIFILM Irvine Scientific varied cell culture media components at different ratios and assessed each ratio for its ability to modulate galactose levels on a model therapeutic Ab. Design of experiment (DoE) analysis was utilized to determine the optimal ratio of components to maximize galactosylation. The optimized formula, named BalanCD Gal Supplement, resulted in the best overall cell growth, titer, and galactosylation compared to the competing glycosylation supplement.

### MATERIALS & METHODS

Fed-batch cultures of Chinese hamster ovary cells expressing an IgG1 Ab against CD20 were grown in BalanCD CHO Growth A (FUJIFILM Irvine Scientific) in 125 mL shake flask vessels. The working volume of the cultures was 30 mL with a seeding density of  $0.3 \times 10^6$  cells/mL. BalanCD CHO Feed 4 was fed to cultures on days 3–7. The BalanCD Gal Supplement was added on day 7 of the cultures.

The competing supplement, when evaluated against the BalanCD Gal Supplement, required addition on days 2–10 every other day when used as directed. Viable cell density and cell viability were assessed by a Beckman Coulter Vi-Cell XR, Ab titer was assessed by a Pall FortéBio QKe, and glycan analysis was assessed by a PerkinElmer LabChip GXII.



**Figure 1. Comparison of galactose-modulating supplements' effect on cell growth and titer.** FUJIFILM Irvine Scientific's BalanCD Gal Supplement and a competing supplement from Supplier 1 were evaluated for their ability to affect (A) cell growth and (B) titer. BalanCD Gal Supplement was able to maintain cell growth and density, and sustain high titers.

## RESULTS

In the DoE investigation, 30 conditions were evaluated in fed-batch cultures. It was found that certain ratios of media compositions were able to reliably control galactosylation levels without compromising cell growth and Ab titers. The DoE analysis resulted in an optimized composition, known as the BalanCD Gal Supplement, which was evaluated against a competing galactose-modulating supplement from another supplier (**Figure 1 and Figure 2**). In these evaluations, the BalanCD Gal Supplement and Supplier 1 supplement were tested in various concentrations. A fed-batch control condition, which had no galactose modulating supplement additions, was also tested for data comparison.

The evaluations resulted in a similar viable cell density (VCD) and cell viability between the control fed-batch culture, the BalanCD Gal Supplement, and the Supplier 1 supplement (**Figure 1**). All the conditions tested showed normalized Ab titer levels similar to the control (**Figure 1**). The glycan profiles were analyzed and all the evaluated conditions were able to raise galactosylation (**Figure 2**). However, when used at 1X supplement concentrations, the BalanCD Gal Supplement was able to achieve 50% galactosylation while the Supplier 1 supplement resulted in 40% (highlighted), demonstrating the improved efficiency of the BalanCD Gal Supplement over the Supplier 1 supplement.

## CONCLUSIONS

In these studies, the optimized formula of the BalanCD Gal Supplement has demonstrated superior efficiency and product quality when tested against a competing galactose modulating supplement from another supplier. The BalanCD Gal Supplement provides users with a greater range of control on product quality using a simplified, low-volume feeding schedule. It is a versatile product that is compatible with a wide range of cell lines and basal growth medium (not all data shown). The BalanCD Gal Supplement is cGMP manufactured, chemically defined, and animal component-free. These features allow it to be easily implemented into either a research or cGMP-ready cell culture process.

## References

1. Hossler P, Khattak SF, Li ZJ. Optimal and consistent protein glycosylation in mammalian cell culture. *Glycobiology*. 2009 Sep; 19(9):936–949.

## Life.Support.

With over 45 years of industry experience, FUJIFILM Irvine Scientific is focused on providing the highest quality cell culture media. We are dedicated to bringing life to products, and ultimately, to the patients who benefit from the resulting therapeutic advances. World-renowned for our unwavering commitment to full-spectrum partnership, FUJIFILM Irvine Scientific has defined the industry standard in support, innovation, and best-in-class turnaround time.

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Condition	Glycan Profile Percentages							Gal%
	M5	G0	G0f	G1	G1f	G2	G2f	
Fed Batch	8.0	11.3	51.6	5.0	17.0	1.6	2.7	17.2
0.5X Gal Suppl	6.5	10.6	40.9	6.2	22.6	2.8	6.1	26.1
1X Gal Suppl	5.8	4.8	17.0	8.9	39.0	5.1	16.5	49.9
1.5X Gal Suppl	6.5	4.4	17.6	8.3	39.5	4.9	16.5	49.7
2X Gal Suppl	6.2	4.1	16.9	8.0	39.7	5.4	17.3	51.0
2.5X Gal Suppl	6.3	4.0	16.5	8.0	39.5	5.0	18.0	51.4
0.5X Supplier 1	7.5	7.8	33.8	7.2	30.6	3.3	8.5	33.6
1X Supplier 1	7.6	6.6	27.1	8.1	33.8	4.3	11.2	40.0
2X Supplier 1	9.2	6.1	18.7	9.9	35.9	5.5	13.2	46.6

**Figure 2. Evaluation of galactose-modulating supplements' ability to affect glycan profile.** FUJIFILM Irvine Scientific's BalanCD Gal Supplement and a competing supplement from Supplier 1 were evaluated in their ability to affect glycan profile. BalanCD Gal Supplement was able to increase Ab galactosylation above that of the competitor's.

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