SAFC Biosciences™
Accelerate Success™

Technical Bulletin

Animal-Component Free Recombinant Human Insulin is Suitable for Use in Serum-Free Media

Introduction

Recombinant human (rHu) insulin is a crucial component in many serum-free cell culture media; responsible for regulating carbohydrate, protein and lipid metabolism in mammalian cells. Previously, SAFC Biosciences' supplier of rHu insulin produced the component in a manner that utilized animal-derived enzymes during the manufacturing process and animal sera in the yeast cell banking process. Given the potential risks associated with products manufactured using animal-derived components, SAFC Biosciences has sought to find acceptable animal-component free substitutes for raw materials whenever possible.

The manufacturer of rHu insulin utilized by SAFC Biosciences is now providing an improved grade of animal-component free recombinant insulin. The improved RHu insulin is manufactured in a newly constructed facility, using processes which are free of animal-derived components. The product is tested according to the European Pharmacopoeia (EP) and complies with EP requirements. In addition, the new insulin has been demonstrated by the manufacturer to be equivalent to the previous grade of insulin. The purpose of these studies was to determine if the new animal-component free rHu insulin product performs equivalently to the previous grade of rHu insulin in our serum-free media.

To demonstrate equivalence, the animal-component free rHu insulin was evaluated in several serum-free media: EX-CELL™ 302, EX-CELL™ 293 and EX-CELL™ VPRO. Media were manufactured in-house using the non-animal-free grade of insulin as the Control, and the new animal-free grade of insulin as the Test. Side by side comparisons were performed using appropriate media, cell lines and culture conditions. Growth was monitored over multiple serial passages and in culture longevity studies in each media. IgG production was also evaluated in EX-CELL™ 302. The performances of the Control and Test media were considered equivalent if cell densities, viabilities, and/or IgG production in the Test medium was $\geq$ 75% of the Control medium.

The results from these experiments indicate that serum-free media containing the new rHu insulin performed in a comparable manner to media prepared with the previous grade of insulin. In each of the Control and Test media evaluated, there was little difference in cell densities, viabilities and IgG production. From these studies, we conclude that the new grade of rHu insulin is an acceptable animal-free substitute component for use in SAFC Biosciences' serum-free media.

Materials

Cells

- B13-24 (DXB11), American Type Culture Collection, ATCC No. CRL-11397
- HEK 293, American Type Culture Collection, ATCC No. CRL-1573
- PER.C6® Crucell, Leiden, The Netherlands

Media, Supplements and Reagents

- EX-CELL™ 302 Serum-Free Medium for CHO Cells, Catalog No. 14312**
- EX-CELL™ 293 Serum-Free Medium for HEK 293 Cells, Catalog No. 14570***
- EX-CELL™ VPRO Serum-Free Medium for Retinoblast Cells, Catalog No. 14560****
- L-Glutamine 200 mM Solution, Catalog No. 59202
- IMMUNO-TEK Quantitative Human IgG Antigen ELISA Kit, ZeptoMetrix Corporation, Buffalo, NY, Catalog No. 0801182

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Methods

Prior to use, all media were supplemented with the following concentrations of L-glutamine:

- EX-CELL™ 302 — 4 mM
- EX-CELL™ 293 — 6 mM
- EX-CELL™ VPRO — 6 mM

All cultures were seeded at 3 x10^5 cells/mL, in either shaker flasks (120 rpm) or roller bottles (1 rpm), and were incubated at 37 C in an 8% CO_2 humidified incubator, and all cultures were passaged every 3 - 4 days. Cell densities and viabilities were determined by trypan blue exclusion using a Cedex Cell Counter (Innovatis, Malvern, PA). IgG production was determined with a human IgG ELISA kit. All studies were performed in triplicate.

EX-CELL™ 302:

Growth in EX-CELL™ 302 was evaluated using DXB11-derived cells. Working stock cultures were seeded in the Control and Test media and evaluated for five passages. Growth curves were generated by sampling cultures daily, without re-feeding for 10 days until viability fell below 70%. Media samples were obtained from the DXB11 cultures over the same 10-day period to evaluate IgG production in the Control and Test media.

EX-CELL™ 293:

Growth in EX-CELL™ 293 was monitored using HEK 293 cells. Working stock cultures of HEK 293 cells were seeded in the Control and Test media and evaluated for six passages. Growth curves were generated by sampling cultures daily for 10 days without re-feeding, until viability fell below 70%.

EX-CELL™ VPRO:

Growth in EX-CELL™ VPRO was examined using a working stock of PER.C6® cells. Cells were seeded in the Control and Test media in roller bottles and evaluated for six passages. Growth curves were generated by sampling cultures daily for nine days without re-feeding, until viability fell below 70%.

Results

Performance in EX-CELL™ 302:

Cell densities and culture viabilities over multiple passages in EX-CELL™ 302 were equivalent in Control and Test media for the DXB11 cell line (Figure 1A). The growth curve demonstrates equivalent growth and viability in the culture longevity studies (growth curves) (Figure 1B). Additionally, no significant difference was noted in the IgG production between the Control and Test media (Figure 1C.). The results in each graph are represented as the average ± the standard deviation.

Performance in EX-CELL™ 293:

Cell densities and culture viabilities over the serial passages and the 10-day growth curve experiments in EX-CELL™ 293 were comparable in both the Control and Test media (Figure 2A and Figure 2B). The results in each graph are represented as the average ± the standard deviation.

Performance in EX-CELL™ VPRO:

Cell densities and culture viabilities over the serial passages and the nine-day growth curve experiments in EX-CELL™ VPRO were comparable in both the Control and Test media (Figure 3A and Figure 3B). The results in each graph are represented as the average ± the standard deviation.

Conclusions

The results from these experiments indicate that media prepared with an improved grade of animal-component free rHu insulin perform in a comparable manner to media prepared with non-animal-component free insulin. In each of the Control and Test media evaluated, there was little difference in cell densities, viabilities and IgG production. Indeed, cell densities and viabilities (and IgG production in EX-CELL™ 302) in each Test media were ≥ 75% of cell densities and viabilities in each Control medium. From these studies, we conclude that the new grade of rHu insulin is an acceptable animal-free substitute component for use in SAFC Biosciences' serum-free media.

*Since this research was conducted, these products are no longer catalog products

**Catalog No. 14312 has been replaced by Catalog No. 14324

***Catalog No. 14570 has been replaced by Catalog No. 14571

****Catalog No. 14560 has been replaced by Catalog No. 14561

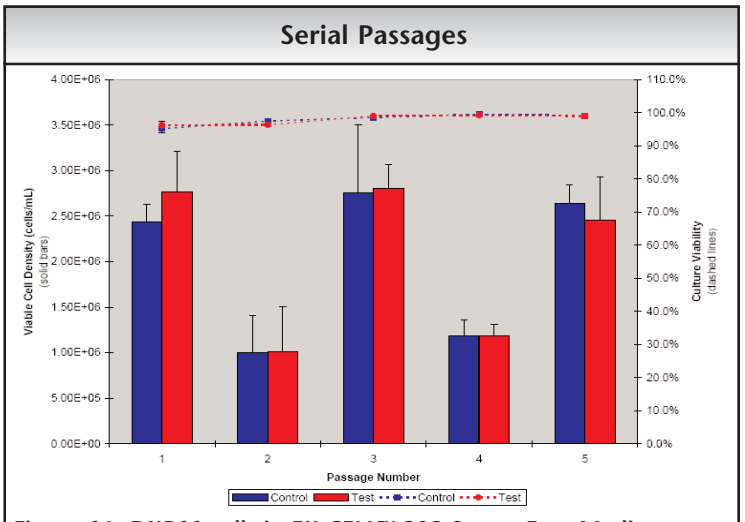

Figure 1A: DXB11 cells in EX-CELL™ 302 Serum-Free Medium.

Growth Kinetics

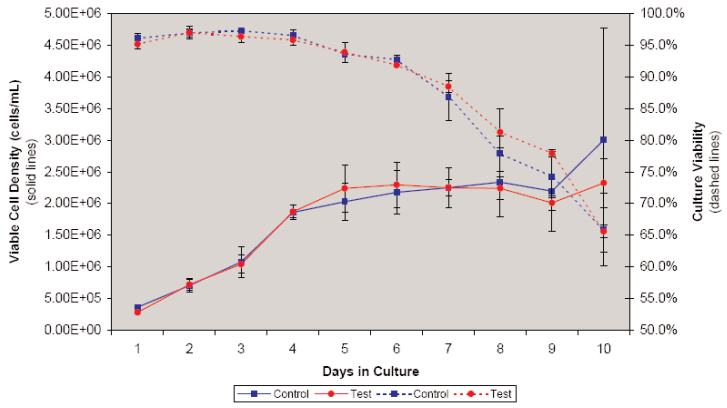

Figure 1B: DXB11 cells in EX-CELL™ 302 Serum-Free Medium.

IgG Production

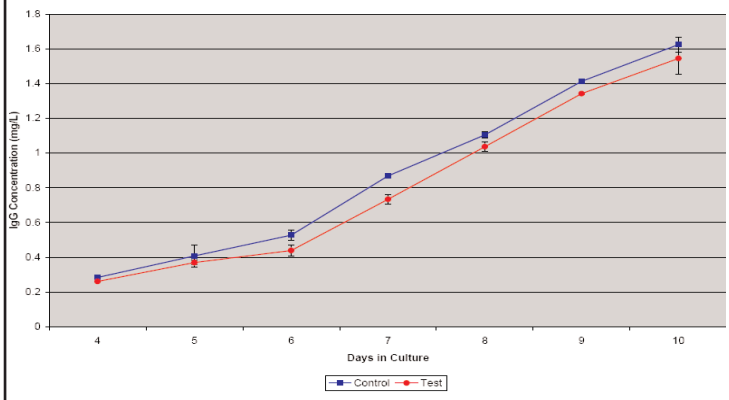

Figure 1C: DXB11 cells in EX-CELL™ 302 Serum-Free Medium.

Serial Passage Growth

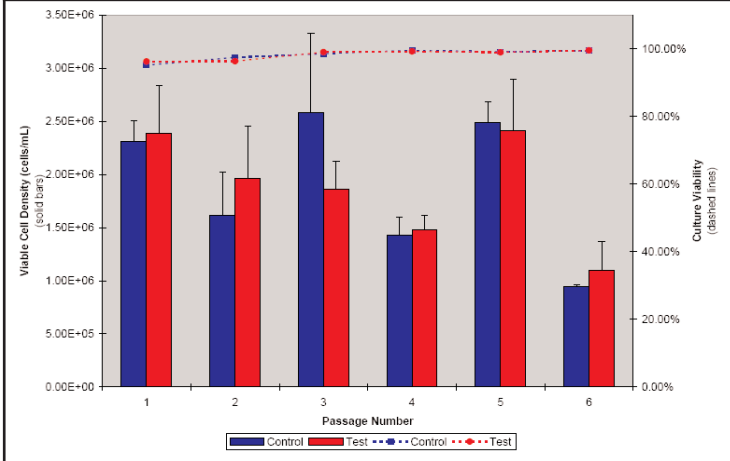

Figure 2A: HEK 293 cells in EX-CELL™ 293 Serum-Free Medium.

Growth Kinetics

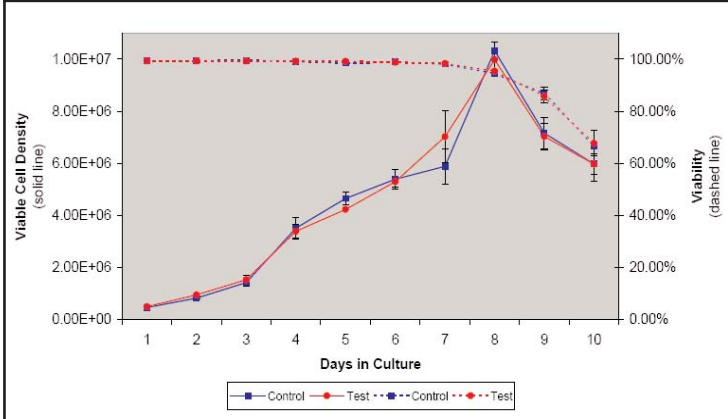

Figure 2B: HEK 293 cells in EX-CELL™ 293 Serum-Free Medium.

Serial Passage Growth

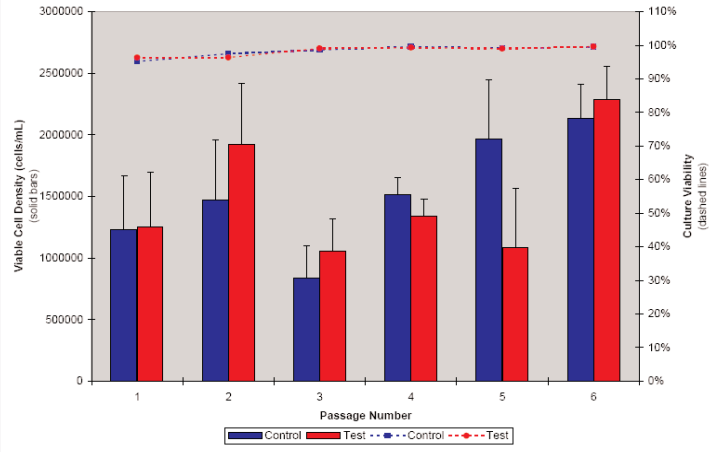

Figure 3A: PER.C6® cells in EX-CELL™ VPRO Serum-Free Medium.

Figure 3B

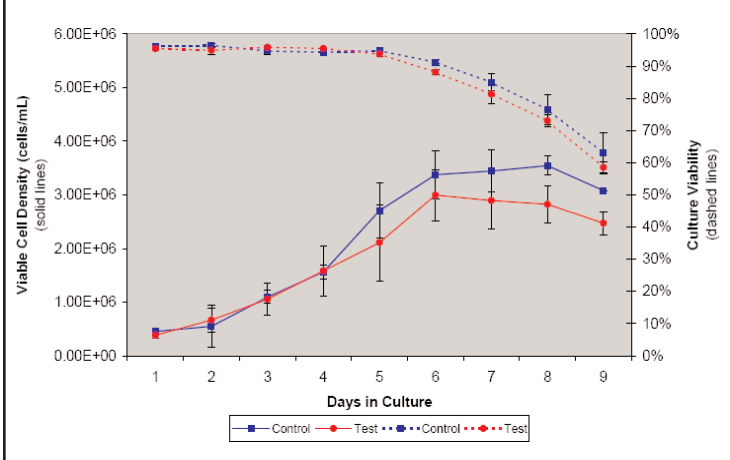

Figure 3B: PER.C6® cells in EX-CELL™ VPRO Serum-Free Medium.

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Issued March 2006 T080
0805 1005

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