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Technical Bulletin

EX-CELL™ CD CHO: A Chemically Defined, Animal-Component Free, Serum-Free Medium for CHO Cells

Introduction

SAFC Biosciences has developed EX-CELL™ CD CHO, a chemically defined, animal-component free, serum-free medium optimized for the growth of CHO-derived cell lines producing recombinant proteins, including monoclonal antibodies (MAb). EX-CELL™ CD CHO is hydrolysate-free and contains no animal-derived components.

The following study was undertaken to demonstrate the ability of EX-CELL™ CD CHO to support the growth and MAb production in a CHO-derived (DXB11) cell line producing human MAb immunoglobulin G (IgG_4). Additionally, the ability of EX-CELL™ CD CHO to support the growth of a non-producing CHO-K1 cell line was assessed. Both cell lines were adapted to EX-CELL™ CD CHO using direct adaptation from serum-containing suspension cultures prior to the evaluation of growth and MAb production.

Materials

Cells

- DXB11, American Type Culture Collection, ATCC No. CRL-11397
- CHO-K1, American Type Culture Collection, ATCC No. CRL-61

Media and Supplements

- EX-CELL™ CD Cho, with hypoxanthine and thymidine, SAFC Biosciences, Catalog No. 14361
- Iscove's Modified Dulbecco's Medium (IMDM), American Type Culture Collection, ATCC No. 30-2005
- Dulbecco's Modified Eagle's Medium/Ham's Nutrient Mixture F12 (DMEM/F12), SAFC Biosciences, Catalog No. 51448
- Fetal Bovine Serum - Gamma Irradiated (FBS), SAFC Biosciences, Catalog No. 12107
- 200 mM L-glutamine, SAFC Biosciences, Catalog No. 59202

Antibody Assay Kit

- Immuno-Tek Quantitative IgG ELISA Kit, ZeptoMetrix Corporation, Catalog No. 0801182

Methods

Media/Supplement Preparation and Storage

EX-CELL™ CD CHO was supplemented with 8 mM L-glutamine at point of use. All media were stored at 2 to 8 C protected from light. Cultures were maintained using aseptic technique with no antibiotic or fungicide supplementation.

Culture Techniques

Prior to adaptation, CHO-K1 and DXB11 cell lines were maintained as suspension cultures in 125 mL shaker flasks in DMEM/F12 (CHO-K1) and IMDM (DXB11) supplemented with 10% FBS and 4 mM L-glutamine. After adaptation to serum-free medium, the cells were transferred to 250 mL shaker flasks and routinely subcultured every 3 - 4 days at a density of 3×10^5 cells/mL and 4×10^5 cells/mL respectively (60 mL volume per 250 mL shaker flask). The flasks were shaken on an orbital shaker at 120 - 130 rpm and were maintained at 37 C in a humidified atmosphere with 10% CO_2. Cell densities and viabilities were determined by trypan blue exclusion.

Adaptation

The CHO-K1 and DXB11 cell lines were adapted to EX-CELL™ CD CHO by direct adaptation. During the first 2 - 3 passages, suspension cultures previously grown in basal medium + 10% FBS were seeded directly into pre-warmed EX-CELL™ CD CHO at a density of 5×10^5 cells/mL. Cells were subcultured every 3 - 4 days and were considered fully adapted when cell densities achieved $> 2.0 \times 10^6$ cells/mL and viabilities of > 95%.

United States
SAFC Biosciences, Inc.
13804 W. 107th Street
Lenexa, Kansas 66215
USA
Phone +1 913-469-5580
Toll free-USA 1 800-255-6032
Fax +1 913-469-5584
E-mail info-na@sial.com

Europe
SAFC Biosciences Ltd.
Smeaton Road, West Portway
Andover, Hampshire SP10 3LF
UNITED KINGDOM
Phone +44 (0)1264-333311
Fax +44 (0)1264-332412
E-mail info-eu@sial.com

Asia Pacific
SAFC Biosciences Pty. Ltd.
18-20 Export Drive
Brooklyn, Victoria 3025
AUSTRALIA
Phone +61 (0)3-9362-4500
Toll free-AUS 1 800-200-404
Fax +61 (0)3-9315-1656
E-mail info-ap@sial.com

www.safcbiosciences.com

Growth Studies and Antibody Production

Growth studies were initiated after adaptation and were monitored over an additional 6 passages. The cells were subcultured one additional time and daily cell counts were determined until culture viabilities dropped below 60%. Additionally, during the last passage, daily aliquots from the DXB11 cell suspension were taken for IgG determination. Each aliquot was micro-centrifuged (1000 rpm) for 2 minutes, followed by collection and storage (-20 C) of the supernatant. Antibody (human IgG) production was determined by ELISA (ZeptoMetrix Human IgG ELISA kit). The appropriate sample dilutions were prepared in dilution buffer supplied with the kit. The absorbance was read at 405 nm on a VersaMax™ microplate reader and calculations were performed using SoftMax® Pro. 4.0 software (both from Molecular Devices Corporation).

Results

Growth Studies

Both the CHO-K1 and DXB11 cell lines typically reached densities of at least 3.5 x 10^6 and 2.5 x 10^6 respectively when subcultured every 4 days in EX-CELL™ CD CHO. Figure 1 depicts the typical growth of CHO-K1 and DXB11 cells over the course of 6 passages in EX-CELL™ CD CHO. Figure 2 illustrates the culture longevity of CHO-K1 and DXB11 cells over a period of 7 - 8 days in EX-CELL™ CD CHO medium. CHO-K1 densities increase steadily over the first 4 days, reach a plateau by day 5 and steadily decline after day 5. Viabilities start to drop after day 5 and are below 60% on day 7. DXB11 densities increase steadily over the first 5 days, reach a plateau by day 6 and decline after day 6. Viabilities start to drop after day 5 and are below 60% on day 7.

Monoclonal Antibody Production

A human IgG ELISA assay kit was used to titer the production of IgG by the DXB11 cell line. Figure 3 depicts a typical viability and concurrent IgG production in the DXB11 cell line cultured in EX-CELL™ CD CHO. Viabilities remain above 90% until day 5 and proceed to decline thereafter. IgG production steadily increases with peak productivity on day 8. Additional evaluations conducted by SAFC Biosciences demonstrate that IgG production in EX-CELL™ CD CHO is comparable to levels typically experienced in a basal formulation + 10% FBS (Figure 4). These studies demonstrate that EX-CELL™ CD CHO supports high density cell growth and MAb production in a CHO-derived cell line.

Figure 1

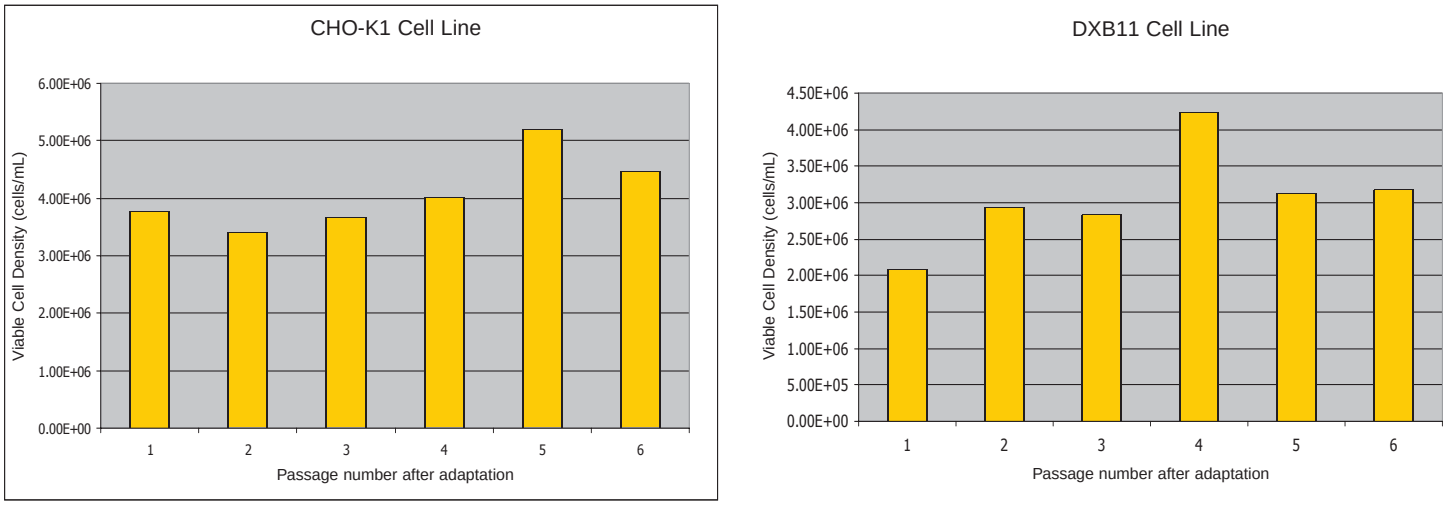
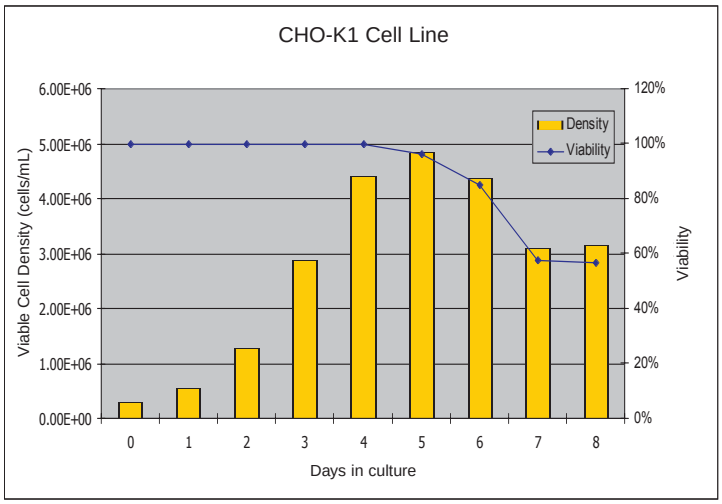


Figure 1: Multiple passage growth of CHO-K1 and DXB11 cells in EX-CELL™ CD CHO.

Figure 2



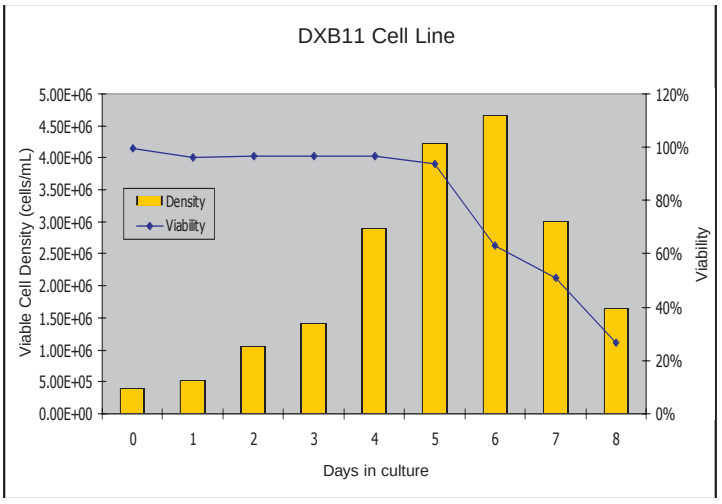


Figure 2: Typical culture longevity of CHO-K1 and DXB11 cells in EX-CELL™ CD CHO.

Figure 3

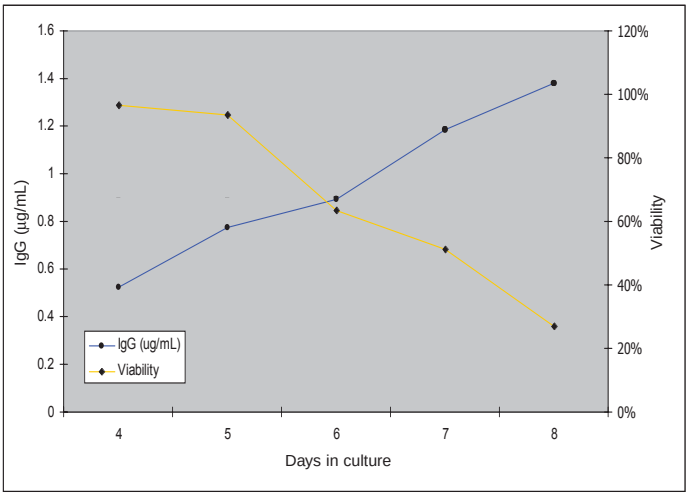


Figure 3: Human IgG production (µg/mL) and cell viability in DXB11 cells cultured in EX-CELL™ CD CHO.

Figure 4

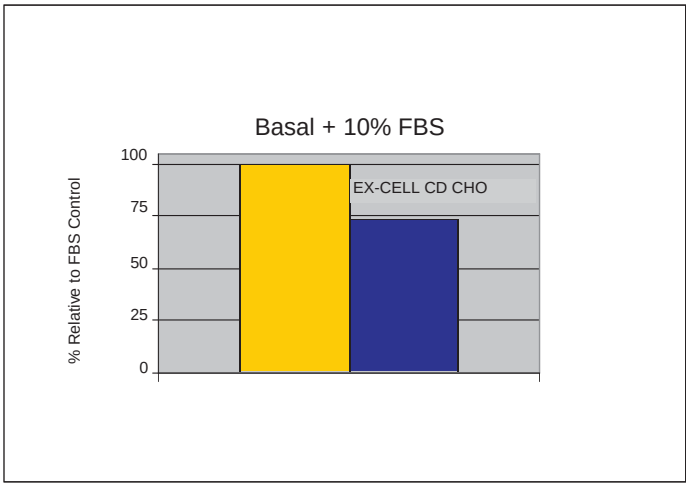


Figure 4: Normalized human IgG production by DXB11 cells cultured in EX-CELL™ CD CHO and IMDM + 10% FBS.

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Issued March 2006 T067
0404 1105

www.safcbiosciences.com

United States

SAFC Biosciences, Inc.
13804 W. 107th Street
Lenexa, Kansas 66215
USA
Phone +1 913-469-5580
Toll free-USA 1 800-255-6032
Fax +1 913-469-5584
E-mail info-na@sial.com

Europe

SAFC Biosciences Ltd.
Smeaton Road, West Portway
Andover, Hampshire SP10 3LF
UNITED KINGDOM
Phone +44 (0)1264-333311
Fax +44 (0)1264-332412
E-mail info-eu@sial.com

Asia Pacific

SAFC Biosciences Pty. Ltd.
18-20 Export Drive
Brooklyn, Victoria 3025
AUSTRALIA
Phone +61 (0)3-9362-4500
Toll free-AUS 1 800-200-404
Fax +61 (0)3-9315-1656
E-mail info-ap@sial.com