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Technical Bulletin

EX-CELL™ NS0: An Animal-Component Free, Protein-Free, Chemically Defined Medium for Monoclonal Antibody Production

Introduction

SAFC Biosciences has developed EX-CELL™ NS0, an animal-component free, protein-free, chemically defined, serum-free medium for growth and monoclonal antibody (MAb) production in murine NS0-derived hybridoma cell lines. EX-CELL™ NS0 is hydrolysate-free and contains no animal- or human-derived components. Additionally, the medium is formulated without L-glutamine to aid in media stability and to avoid L-glutamine degradation and ammonia build-up.

The following study was undertaken to demonstrate the ability of EX-CELL™ NS0 to support growth and MAb production in two NS0-derived hybridoma cell lines: the SC-71 cell line which secretes the mouse MAb immunoglobulin G (IgG_1) and BA-D5 which secretes IgG_{2b}. The cell lines were adapted to EX-CELL™ NS0 by direct adaptation and growth studies and MAb production were assessed.

We conclude that EX-CELL™ NS0 supports hybridoma cell growth and supports greater MAb production in comparison with serum-supplemented cultures.

Materials

Cells

- SC-71, American Type Culture Collection, ATCC No. HB-277
- BA-D5, American Type Culture Collection, ATCC No. HB-287

Media and Supplements

- EX-CELL™ NS0, SAFC Biosciences, Catalog No. 14650
- Lipid Concentrate (500X), Chemically Defined, SAFC Biosciences, Catalog No. 14100
- 200 mM L-glutamine, SAFC Biosciences, Catalog No. 59202
- Dulbecco's Modified Eagle's Medium (DMEM/High Modified), SAFC Biosciences, Catalog No. 51444
- Fetal Bovine Serum - Gamma Irradiated (FBS), SAFC Biosciences, Catalog No. 12107

Antibody Assay Kit

- Easy-Titer® Mouse IgG Assay Kit, Pierce Biotechnology, Catalog No. 23300
- Mouse IgG Standard, Pierce Biotechnology, Catalog No. 31204

Methods

Media/Supplement Preparation and Storage

Prior to use, EX-CELL™ NS0 was supplemented with 8 mM L-glutamine and 1X Lipid Concentrate (500X) (1:500 dilution). All media was stored at 4 C protected from light. Other supplements were stored at their recommended temperatures. Cultures were maintained using aseptic technique with no antibiotic or fungicide supplementation. L-glutamine was added at point of use.

Culture Techniques

Prior to adaptation, SC-71 and BA-D5 cell lines were maintained as static cultures in 75 cm^2 T-flasks in DMEM supplemented with 10% FBS and 4 mM L-glutamine. After adaptation to serum-free media in shaker flasks, the cells were routinely subcultured every three days at a seeding

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density of 1 x 10^5 cells/mL and 2 x 10^5 cells/mL respectively (30 mL volume per 125 mL shaker flask). The flasks were shaken on an orbital shaker at 110 rpm and were maintained at 37 C in a humidified incubator with 5% CO_2. Cell densities and viabilities were determined by trypan blue exclusion.

Growth Studies and Antibody Production

The SC-71 and BA-D5 cell lines were adapted to EX-CELL™ NS0 by direct adaptation. Briefly, cultures previously growing in DMEM + 10% FBS were seeded directly into pre-warmed serum-free media at the seeding densities previously mentioned. Cells were subcultured every three days and were considered fully adapted after 6 passages (18 days in each serum-free medium). Growth studies were initiated at the seventh subculture and were monitored over an additional 4 passages. The cells were subcultured one more time and daily cell counts were taken during the last passage. Additionally, during the last passage, daily aliquots from each cell suspension were taken for IgG determination. Each aliquot was micro-centrifuged (1000 rpm) for 2 minutes, and then the supernatant was removed, transferred to a new tube and frozen at -20 C. Antibody (mouse IgG) production was determined by ELISA (Easy-Titer Mouse IgG Assay Kit) with Mouse IgG used to generate the standard curve. 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

Adaptation and Growth Studies

During this study a new serum-free hybridoma medium, EX-CELL™ NS0, was evaluated for growth and antibody production using the hybridoma cell lines SC-71 and BA-D5. Both cell lines adapted extremely well to EX-CELL™ NS0, achieving cell densities of greater than 1.5 x 10^6 cells/mL and viabilities greater than 90% by the third passage. Figure 1 illustrates the typical growth of SC-71 and BA-DA cells in EX-CELL™ NS0 in comparison with DMEM + 10% FBS over multiple subcultures. Figure 2 depicts typical SC-71 and BA-D5 growth curves in each media. The average cell densities, viabilities and doubling times (attained on day 3 post-subculture) in each medium are in the following table.

Cell Line	Medium	Average Cell Density (Cells/mL)	Average % Viability	Average Doubling Time (Hours)
SC-71	EX-CELL™ NS0	2.2 x 10^6	95.5	16.1
SC-71	DMEM + 10% FBS	1.2 x 10^6	84.5	20.0
BA-D5	EX-CELL™ NS0	2.9 x 10^6	92.0	18.6
BA-D5	DMEM + 10% FBS	1.9 x 10^6	92.6	22.2

Monoclonal Antibody Production

A mouse IgG ELISA assay kit was used to titer the production of IgG. Figure 3 illustrates IgG production in both the SC-71 and BA-D5 cell lines in EX-CELL™ NS0 and DMEM + 10% FBS. Antibody production by SC-71 cells growing in EX-CELL™ NS0 was approximately 2-fold higher than in the serum control in the same cell line. BA-D5 cells in EX-CELL™ NS0 produced an approximately 3-fold increase in titer over the serum control. These studies indicate that EX-CELL™ NS0 supports high-density cell growth and MAb production in NS0-derived hybridoma cell lines.

Figure 1

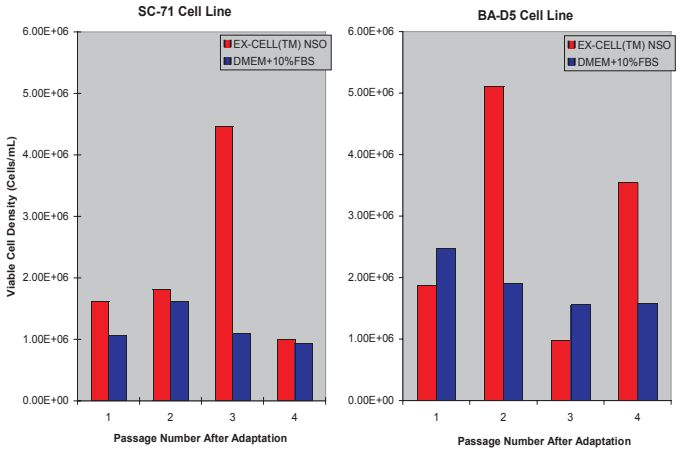

Figure 1: Multiple-passage growth of SC-71 and BA-D5 cells in EX-CELL™ NS0 and DMEM + 10% FBS.

Figure 2

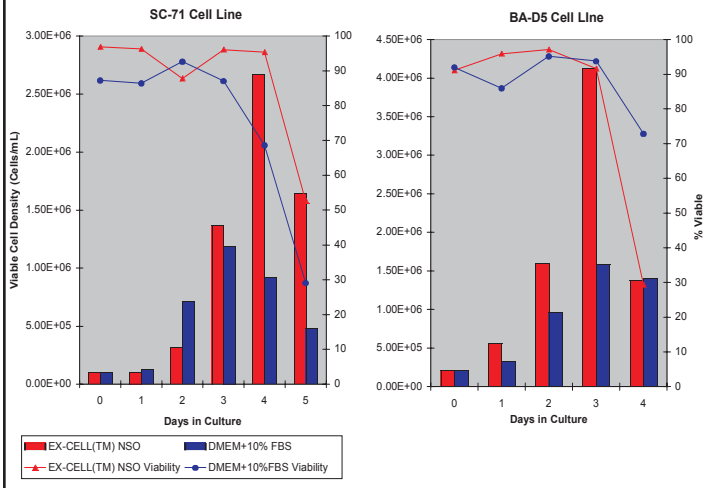

Figure 2: Typical growth curves of SC-71 and BA-D5 cells in EX-CELL™ NS0 and DMEM + 10% FBS.

Figure 3

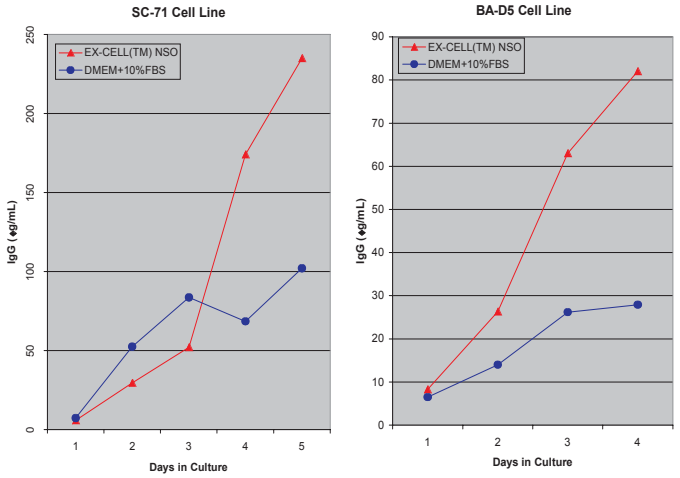

Figure 3: Mouse IgG production (µg/mL) by SC-71 and BA-D5 cells cultured in EX-CELL™ NS0 and DMEM + 10% FBS.

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