

Research Report

EX-CELL™ NS0: An Animal-Component Free, Protein-Free, Chemically Defined Medium for Monoclonal Antibody Production in Murine NS0 Hybridoma Cell Lines

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Introduction

The demand for hybridoma cell lines expressing highly specific monoclonal antibodies (MAbs) has increased dramatically in recent years due to increased needs for MAbs used in diagnostic assays and as novel therapeutic agents. Recent advances in serum-free media and bioreactor systems make murine-derived hybridoma cell lines a practical and cost-effective approach to large-scale MAb production. The advantages of using a serum-free system include availability of extremely effective chemically defined, regulatory compliant media; ease of adaptation and scale-up in the media; consistent control over MAb production levels; ease in purification; and less utilization of animals and animal-derived products.

SAFC Biosciences has developed EX-CELL™ NS0, a chemically defined, serum-free medium for growth and MAb production in NS0-derived hybridoma cell lines. EX-CELL™ NS0 media is protein-free, hydrolysate-free and contains no animal- or human-derived components. Additionally, the medium is formulated without L-glutamine to aid in 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 mouse immunoglobulin G (IgG₁) and BA-D5 which secretes IgG_{2b}. The cell lines were adapted to EX-CELL™ NS0 and GIBCO™ CD Hybridoma Medium and growth studies and MAb production were evaluated. We conclude that both EX-CELL™ NS0 and GIBCO™ CD Hybridoma Medium support hybridoma cell growth in a comparable manner and outperform serum-supplemented cultures. Dependent upon the cell line, EX-CELL™ NS0 either outperforms or is comparable to GIBCO™ CD Hybridoma Medium for MAb production.

Materials

Cells

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

Serum-Free Media

- EX-CELL™ NS0, SAFC Biosciences, Inc., Catalog No. 14650
- Lipid Concentrate (500X), Chemically Defined, SAFC Biosciences, Inc., Catalog No. 14100
- GIBCO™ CD Hybridoma Medium, Invitrogen Corporation, Catalog No. 11279023
- GIBCO™ 250X Cholesterol Lipid Concentrate, Invitrogen Corporation, Catalog No. 12531018

Other Media and Supplements

- L-glutamine, 200 mM, SAFC Biosciences, Inc., Catalog No. 59202
- Dulbecco's Modified Eagle's Medium (DMEM/High Modified), SAFC Biosciences, Inc., Catalog No. 51444
- Fetal Bovine Serum - Gamma Irradiated (FBS), SAFC Biosciences, Inc., 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). CD Hybridoma Medium was supplemented with 8 mM L-glutamine and 1X Cholesterol Lipid Concentrate (250X), (1:250 dilution). All media were 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.

Culture Techniques

Prior to adaptation, SC-71 and BA-D5 cell lines were maintained as static cultures in 75 cm² T-flasks in DMEM supplemented with 10% FBS and 4 mM L-glutamine. After adaptation to each serum-free media in shaker flasks, the cells were routinely subcultured every three days at a seeding density of 1 x 10⁵ cells/mL to 2 x 10⁵ cells/mL

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(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% CO₂. 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 each serum-free medium 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 (P7 - 10). The cells were subcultured one more time and daily cell counts were taken during the last passage (P11). 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, two serum-free formulations (EX-CELL[™] NS0 and CD Hybridoma Medium) were evaluated for growth and antibody production using the hybridoma cell lines SC-71 and BA-D5. Both cell lines adapted extremely well in EX-CELL[™] NS0 and CD Hybridoma Medium, and growth and viabilities were robust. Both serum-free media supported cell growth better or comparable to serum-supplemented cultures.

Figure 1 illustrates the typical growth of SC-71 and BA-D5 cells in EX-CELL[™] NS0, CD Hybridoma Medium and DMEM + 10% FBS over multiple subcultures. Both cell lines reached higher cell densities in EX-CELL[™] NS0 and CD Hybridoma Medium as compared to serum-supplemented control.

Figure 2 depicts typical SC-71 and BA-D5 growth kinetic curves in each media. The figure illustrates that EX-CELL[™] NS0 compared to CD Hybridoma Medium can exhibit significantly higher cell densities. Cell densities in EX-CELL[™] NS0 were more than double for the SC-71 cell line by day 4 and at least 30% more for BA-D5 cell line on day 3.

The average cell densities, viabilities and doubling times (attained on day 3 post-subculture) in each medium are shown in Table 1.

Monoclonal Antibody Production

The IgG production in both cell lines was measured by a commercially available ELISA assay. Figure 3 illustrates IgG production for both the SC-71 and BA-D5 cell lines in EX-CELL[™] NS0 and CD Hybridoma Medium and DMEM+10% FBS. EX-CELL[™] NS0 supported MAb production to more than double as compared to serum-supplemented control.

MAb production in BA-D5 cells was comparable in both serum-free formulations on day 4, and it was approximately 3-fold higher than the serum control. MAb levels were more than double in EX-CELL[™] NS0 as compared to CD Hybridoma Medium for the SC-71 cell line.

Conclusions

- SC-71 and BA-D5 hybridoma cell lines adapted easily to EX-CELL[™] NS0 and CD Hybridoma Medium.
- Growth characteristics (cell density and viability) of both cell lines were comparable in EX-CELL[™] NS0 and CD Hybridoma Medium to the serum controls.
- MAb production by SC-71 cells was approximately 2 times greater in EX-CELL[™] NS0 than in CD Hybridoma Medium and the serum control on day 4.
- MAb production by BA-D5 cells was approximately 3 times greater in EX-CELL[™] NS0 and CD Hybridoma Medium than the serum control.

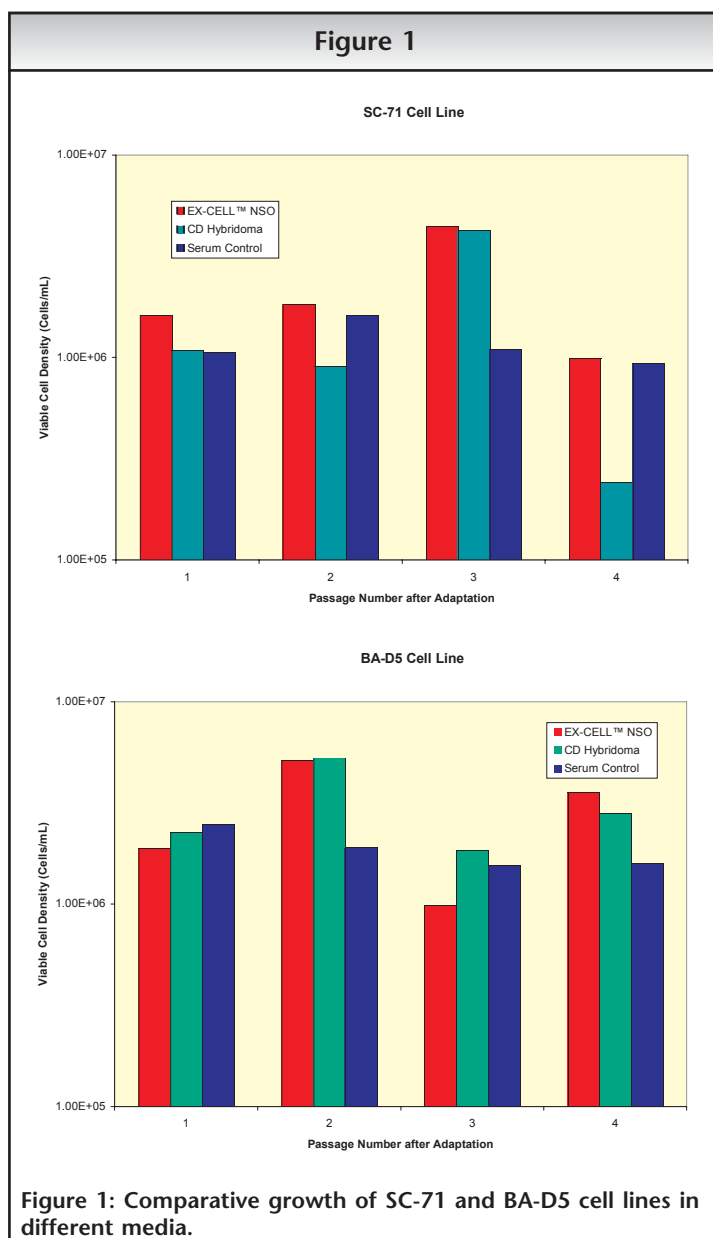


Figure 2

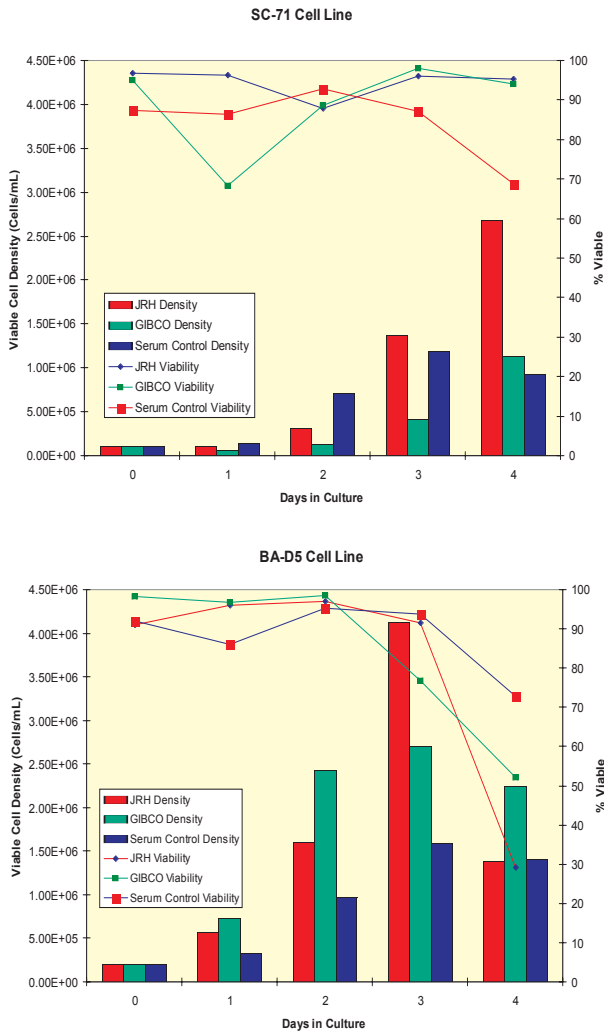


Figure 2: Comparative growth kinetics of SC-71 and BA-D5 cell lines in different media.

Figure 3

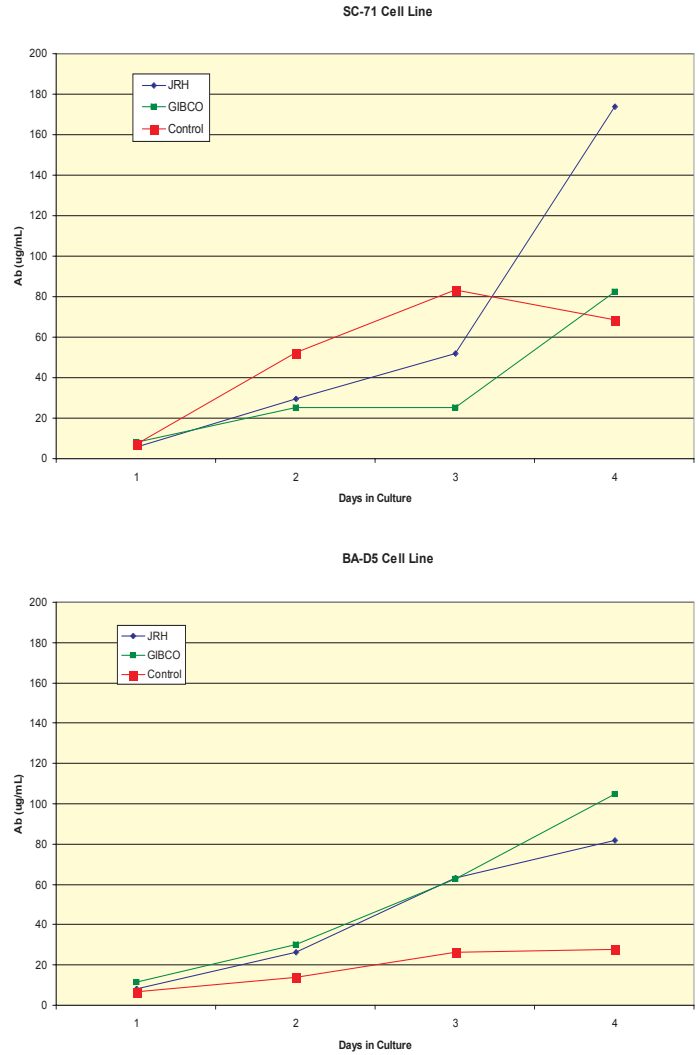


Figure 3: Comparative IgG production in SC-71 and BA-D5 cell lines in different media.

Table 1

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	CD Hybridoma	1.6 x 10 ⁶	94.1	17.9
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	CD Hybridoma	3.0 x 10 ⁶	89.8	18.4
BA-D5	DMEM + 10% FBS	1.9 x 10 ⁶	92.6	22.2

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