SAFC Biosciences™
Accelerate Success™

Technical Bulletin

EX-CELL™ Sp2/0: An Animal-Component Free, Protein-Free, Chemically Defined Medium for Monoclonal Antibody Production

Introduction

SAFC Biosciences has developed EX-CELL™ Sp2/0 Serum-Free Medium for Sp2/0 Cells, an animal-component free, protein-free, chemically defined, serum-free medium for growth and monoclonal antibody (MAb) production in Sp2/0-derived hybridoma cell lines. EX-CELL™ Sp2/0 is hydrolysate-free and contains no animal-or human-derived components.

The following study was undertaken to demonstrate the ability of EX-CELL™ Sp2/0 to support growth and MAb production in the mSxl 5 cell line (an Sp2/0-derived hybridoma) that secretes mouse MAb immunoglobulin G (IgG_1). The cell line was adapted to EX-CELL™ Sp2/0 by direct adaptation and growth and MAb production were assessed.

Materials

Cells

- mSxl 5, American Type Culture Collection, ATCC No. CRL-1951

Media and Supplements

- EX-CELL™ Sp2/0, SAFC Biosciences, Catalog No. 14660
- L-Glutamine Solution 200 mM, SAFC Biosciences, Catalog No. 59202
- RPMI 1640 Medium, SAFC Biosciences, Catalog No. 51502
- Fetal Bovine Serum - Gamma Irradiated (FBS), SAFC Biosciences, Catalog No. 12107

Antibody Determination

- HPLC gel permeation column, ZORBAX GF-450, Agilent Technologies

Methods

Media/Supplement Preparation and Storage

Prior to use, EX-CELL™ Sp2/0 was supplemented with 8 mM L-glutamine on day zero. All media were stored at 2 to 8 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

The mSxl 5 cells were cultured in 125 mL Erlenmeyer shaker flasks (30 mL media volume) at 165 rpm in a 37 C humidified incubator with 10% CO_2. Cultures were initiated in RPMI 1640 + 5% FBS and were subcultured every three days at a seeding density of 3 x 10^5 cells/mL. The cultures were then directly adapted (without weaning) to EX-CELL™ Sp2/0 supplemented with L-glutamine, using the same culture conditions as described above. Cultures were monitored over multiple passages to assess growth kinetics and viability during and after the adaptation period. Cell counts and viability were determined daily by trypan blue exclusion and small aliquots of supernatant were also removed daily for IgG analysis. IgG concentration was determined by HPLC using ImmunoPure Mouse IgG as the standard.

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

Results

Adaptation and Growth Studies

The mSxl 5 cells were adapted directly from RPMI 1640 Medium + 5% FBS into EX-CELL™ Sp2/0. Figure 1 illustrates direct adaptation. During the first passage in EX-CELL™ Sp2/0 cell counts were approximately 25 - 50% lower than average cell densities at each pass; however, they quickly recovered and displayed normal densities and viabilities thereafter. The mSxl 5 cells typically reached densities of 1.5-2.5 x 10^6 cells/mL and exhibited doubling times of 20 - 30 hours when subcultured every three days in EX-CELL™ Sp2/0 (Figure 1).

Figure 2 shows growth kinetics of mSxl 5 cells over a period of six days in EX-CELL™ Sp2/0 media. The cell densities per mL increase steadily over the first four days, reach a plateau by day five and decline after day five. Viabilities start to drop after day four and are below 70% on day six.

A typical viability and concurrent IgG production of adapted mSxl 5 cells in EX-CELL™ Sp2/0 is shown in Figure 3. Viabilities stay above 90% until day four and start to decline thereafter. IgG production steadily increases with peak productivity on days five and six.

Conclusions

SAFC Biosciences developed an animal-component free, protein-free, chemically defined, serum-free medium that supports the growth of mSxl 5 cells in suspension cultures, attaining densities of 2.5 x 10^6 cells/mL. Monoclonal antibody production remains in the 160 mg/L range at day five while viabilities have started to decline. EX-CELL™ Sp2/0 supports Sp2/0-derived hybridoma cell growth and MAb production.

Figure 1

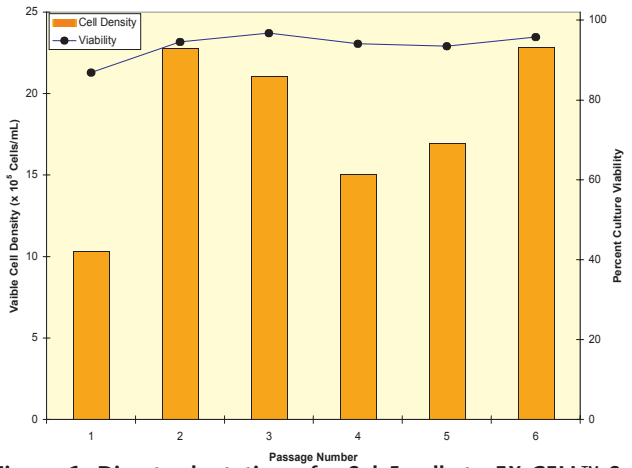

Figure 1: Direct adaptation of mSxl 5 cells to EX-CELL™ Sp2/0 from RPMI 1640 + 5% FBS.

Figure 2

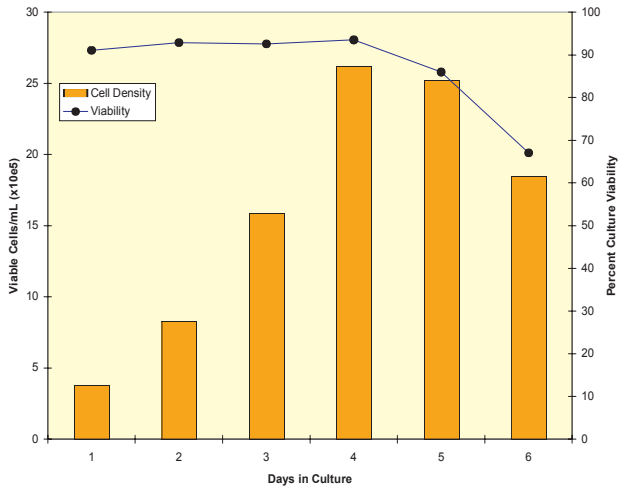

Figure 2: mSxl 5 cell growth and viability over six days.

Figure 3

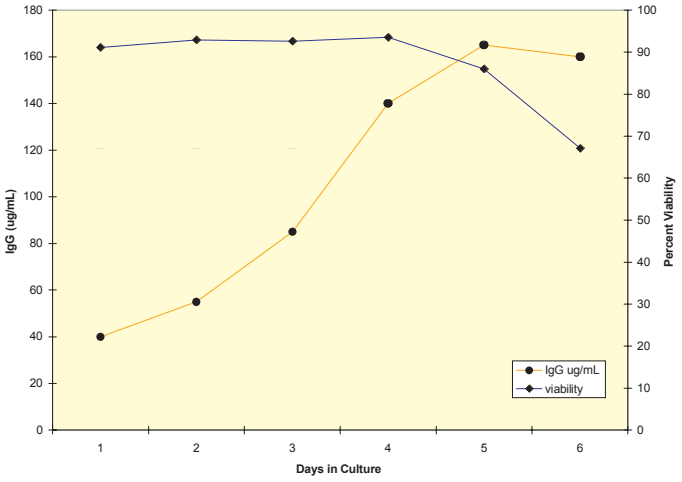

Figure 3: mSxl 5 cell viability and IgG production in EX-CELL™ Sp2/0 in shaker flasks.

Warranty, Limitation of Remedies

SAFC Biosciences warrants to the purchaser for a period of one year from date of delivery that this product conforms to its specifications. Other terms and conditions of this warranty are contained in SAFC Biosciences' written warranty, a copy of which is available upon request. ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING THE IMPLIED WARRANTY OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, ARE EXCLUDED. In no case will SAFC Biosciences be liable for any special, incidental, or consequential damages arising out of this product or the use of this product by the customer or any third party based upon breach of warranty, breach of contract, negligence, strict tort, or any other legal theory. SAFC Biosciences expressly disclaims any warranty against claims by any third party by way of infringement or the like. THIS PRODUCT IS INTENDED FOR PURPOSES DESCRIBED ONLY AND IS NOT INTENDED FOR ANY HUMAN OR THERAPEUTIC USE.

Additional Terms and Conditions are contained in the product Catalog, a copy of which is available upon request.

Issued March 2006 T062
0603 08051105

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