UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF SERUM FREE MEDIUM FOR THE PRODUCTION OF HUMANIZED ANTI-CEA MONOCLONAL ANTIBODY

NURZILA BT. AB. LATIF

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OPTIMIZATION OF SERUM FREE MEDIUM FOR THE PRODUCTION OF HUMANIZED ANTI-CEA MONOCLONAL ANTIBODY

By

NURZILA BT. AB. LATIF

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

December 2011

Dedicated for my beloved family for their love and encouragement

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

OPTIMIZATION OF A SERUM FREE MEDIUM FOR THE PRODUCTION OF HUMANIZED ANTI-CEA MONOCLONAL ANTIBODY

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December 2011

Chair: Professor Tey Beng Ti, PhD

Institute: Bioscience

The humanized monoclonal antibody (mAb) against the colon and ovarian cancer cells has been successfully expressed in NS0 cell line. This humanized mAb can be used to detect and treat human colorectal cancer. Historically, medium supplemented with serum were widely used in animal cell culture. However, the used of serum may leads to virus and prion contamination and gives problems for the downstream process due to its unknown and undefined component. The aim of this study is to develop a suitable serum free culture medium for the production of the humanized mAb. Two types of basal medium, DMEM and IMDM were tested as suitable basal medium for the growth of NS0 cell. Cells were cultured in both basal medium supplemented with different concentration of fetal bovine serum (FBS) (1, 3, 5, 7, 10 (v/v)), 4mM of L-glutamine and 10 μg/ml of antibiotic. The cell concentration and viability of the NS0 cells were determined using tryphan blue exclusion method and ELISA was used to determine the mAb titers of the cultures. The results showed that the higher growth and mAb productivity were observed in DMEM supplemented with 10 % (v/v) FBS. The concentration reached 4.76 x 10^6 cells/mL compared to only 1.95 x 10^6 cells/mL in IMDM supplemented with 10 % (v/v) FBS medium. The

highest antibody titer, 12.89 μg/ml, was obtained from culture condition of DMEM supplemented with 7 % (v/v) FBS. The other objective of this study is to optimize a serum free medium for the NS0 cell line. Different ratios of DMEM/Ex-cell medium were studied. Results showed that the most suitable ratio for the cell line to grow and producing antibody is in 5:5 DMEM/Ex-cell medium. In order to replace serum in cell culture medium, three component; insulin, SyntheChol and L-glutamine were tested using design of experiment (DOE). By using the 2 level factorial design screening method, the significant of the parameters are determined. Then further optimization was done using Central Composite Design (CCD) by augmenting the previous design using 8 additional runs. Experiments were done in T-flask with working volume of 5 mL. Results showed that several combinations gave good but not an optimum result for the responses. This is due to the narrow ranges of parameters that have been used during experimenting. A larger scale of culture (50 mL) using the optimize condition suggested by the software has substantially increased the viable cell concentration and antibody production by 75 % and 48 %, respectively compare to the non-optimal condition.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGOPTIMUMAN MEDIUM BEBAS SERUM UNTUK PENGHASILAN ANTIBODI MONOKLON ANTI-CEA DISESUAIKAN

Oleh

NURZILA BT. AB. LATIF

Disember 2011

Pengerusi: Profesor Tey Beng Ti, PhD

Institut: Biosains

Antibodi monoklon (mAb) disesuaikan yang digunakan untuk membantu dalam proses mengenalpasti sel-sel kanser telah berjaya dihasilkan dalam sel NS0. MAb disesuaikan ini boleh digunakan untuk mengenalpasti serta merawat kanser kolon. Pada kebiasaannya, serum telah ditambahkan ke dalam media kultur sel haiwan secara meluas. Walau bagaimanapun, penambahan serum boleh membawa kepada kontaminasi daripada virus dan prion serta memberi masalah kepada proses akhir; kesan daripada komponen-komponen yang tidak dikenalpasti yang terdapat dalam serum. Tujuan kajian ini adalah untuk memformulasi satu media kultur sel tanpa serum yang sesuai untuk penghasilan mAb daripada sel NS0. Dua jenis media asas, DMEM dan IMDM telah diuji untuk menentukan medium yang sesuai untuk pertumbuhan sel NS0. Sel NS0 telah dikultur di dalam kedua-dua media asas tersebut dan ditambah serum fetus lembu (FBS) dengan kepekatan yang berbeza (1, 3, 5, 7, 10 % (v/v)) berserta 4 mM L-glutamin dan 10 μg/mL antibiotik. Kepekatan serta pertumbuhan sel NS0 ditentukan menggunakan kaedah penyisihan tripan biru manakala ELISA telah digunakan untuk menentukan kepekatan mAb yang terhasil. Keputusan kajian menunjukkan bahawa pertumbuhan sel NS0 dan produktiviti mAb adalah lebih tinggi dalam media DMEM yang ditambahkan dengan 10 % (v/v) FBS.

Jumlah sel yang diperoleh adalah 4.76 x 10^6 sel/mL berbanding hanya 1.95 x 10^6 sel/mL dalam IMDM yang ditambahkan dengan 10 % (v/v) FBS. Titer antibodi tertinggi, 12.89 μg /ml telah diperoleh daripada sel yang dikultur dalam DMEM yang ditambahkan dengan 7 % (v/v) FBS. Keputusan kajian ini menunjukkan bahawa kepadatan sel dan pengeluaran mAb yang tinggi boleh dicapai dengan mengkultur sel dalam media asas yang sesuai. Kajian ini turut bertujuan untuk mengoptimumkan medium bebas serum untuk sel NS0. Campuran nisbah yang berlainan antara medium DMEM dan Ex-cell dikaji. Keputusan menunjukkan nisbah yang paling sesuai untuk sel NS0 hidup dan menghasilkan antibodi adalah dalam nisbah 5:5 DMEM/ Ex-cell. Dalam usaha untuk menggantikan serum dalam medium kultur sel, tiga komponen; insulin, SyntheChol dan L-glutamin telah diuji dengan menggunakan reka bentuk eksperimen (DOE). Dengan menggunakan reka bentuk faktoran 2 peringkat, parameter-parameter bermakna ditentukan. Kemudian, pengoptimuman lanjut telah dilakukan menggunakan Reka Bentuk Pusat Komposit (CCD) dengan menambah lapan eksperimen kepada reka bentuk eksperimen sebelumnya. Eksperimen-eksperimen telah dijalankan dalam kelalang T dengan isipadu kultur sebanyak 5 mL. Keputusan yang diperoleh menunjukkan bahawa beberapa gabungan faktor memberikan keputusan yang baik tetapi ia bukan merupakan hasil yang optimum. Ini adalah kerana julat parameter-parameter yang kecil telah digunakan semasa menjalankan eksperimen. Kultur tisu berskala besar dengan menggunakan nilai-nilai optimum yang telah dicadangkan oleh perisian komputer telah meningkatkan kepekatan sel sebanyak 75 % dan pengeluaran antibodi sebanyak 48 % berbanding sebelum keadaan optimum digunapakai.

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I certify that an Examination Committee has met on **date of viva voce** to conduct the final examination of **Nurzila Bt. Ab. Latif** on her Master thesis entitled **"Optimization of a serum free medium for the production of humanized anti-CEA monoclonal antibody"** in accordance with the Universities and University College Act 1971 and Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Syahida Ahmad, PhD
Faculty of Biotechnology & Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Rosfarizan Mohamad, PhD
Assoc. Professor
Faculty of Biotechnology & Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Muhajir Hamid, PhD
Assoc. Professor
Faculty of Biotechnology & Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Fadzilah Adibah Abd. Majid, PhD
Assoc. Professor
Faculty Chemical Engineering
Universiti Teknologi Malaysia
(External Examiner)

_____

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of **Master of Science.** The members of the Supervisory Committee were as follows:

Tey Beng Ti, PhD
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

Norhafizah Abdullah, PhD
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

Badarulhisam Abdul Rahman, PhD
Adjunct Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

_____

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NURZILA BT. AB. LATIF

Date: 2 December 2011

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