

An Effective Strategy for Recombinant CHO IgG Cell Line and Cell Culture Process Development

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Introduction

Several recombinant CHO IgG clones that can stably express mAb at an industrially acceptable level were developed and expanded by the Viropro-BRI team based on their production levels and cell viabilities using CD CHO as the base medium in batch cultures with cumate induction.

Invitrogen selected one of the best recombinant CHO IgG clone and optimized cell culture feeds and process in a very short development time (<2 months), for both high density cell growth and maximal IgG production applying simple fed-batch development protocols. The fed-batch strategies used chemically defined CD CHO or CD OptiCHO™ media as the base media and two chemically defined feeds, CHO CD EfficientFeed™ A and B, and mixtures of A and B at different feeding volumes and different feeding schedules.

Materials & Methods

Media and Feed

Chemically defined media, CD CHO Medium and CD OptiCHO™ Medium were used as base media.

CHO CD EfficientFeed™ A and B were used for fed-batch development. Universal nutrient requirements for cell growth and recombinant protein expression were considered for designing the feeds.

Cells

We have generated recombinant CHO cell line that harbor elements of the inducible cumate system, allowing a cultivation protocol wherein cells are not burdened with protein production during the proliferative phase and minimizing the perturbations to the host cell when the expressed protein is not well tolerated. This technology broadens the spectrum of proteins that can be stably expressed.

Establishing Suspension Culture

A stepwise adaptation protocol was to recover cells from cryopreservation. The recombinant CHO IgG clones were recovered from cryopreservation as static culture in CD CHO supplemented with 4 mM L-glutamine and 50 µg/ml dextran sulfate. When cell number reached 1-1.5 x 10⁶ cells/ml, cells were diluted to 1:1 ratio with fresh CD CHO. When viability reached 90%, cell culture was passed into media containing 600 µg/ml hygromycin. Cultures were incubated at 37°C, 5% CO₂. Suspension culture was established at passage 4 with seeding density of 0.3x10⁶ cells/ml and shaken at 110 rpm. From the next passage, it was established gradually suspension culture in CD OptiCHO™ (starting with 50%:50% and then 100%).

Induction by Cumate

Recombinant CHO clone # 958 was seeded into duplicate 30 ml cultures at 0.75 x10⁶ cells/ml in the CD CHO and CD OptiCHO™ media without hygromycin. After incubation for 48 hrs at 37°C, the cells were switched to the "on" conditions by adding 1 µg cumate per one milliliter culture and then all of cell conditions were transferred to 30°C, 5% CO₂, 110 rpm.

Feeding Strategies

Different feeding times, feed concentrations and mixtures of the CHO CD EfficientFeed™ A and B were utilized to improve IgG productivity and maintain high cell viability.

Assay

Viable cell densities were determined using a VICELL™, automated cell counting system.

IgG concentrations were established using FortéBio Octet, a novel system that measures the binding rate to a sensor surface coated with protein A.

Expression System

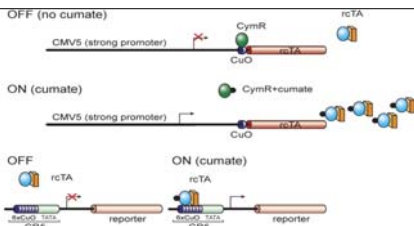


Figure 1. Schematic representation of the expression strategy. The expression of rTA is controlled by the CMV5-CuO promoter. In the absence of cumate, CymR binding to CuO blocks the synthesis of rTA. In the absence of rTA, GFP expression from CR5-GFP is not stimulated. Leaky expression of rTA is not sufficient to activate transcription from the CR5 promoter. Upon addition of 1 µg/ml cumate, CymR binding to CuO is abrogated and therefore rTA is synthesized. Cumate binding to rTA activates its DNA binding activity thereby turning on reporter gene expression.

Clones Expressing Recombinant mAb

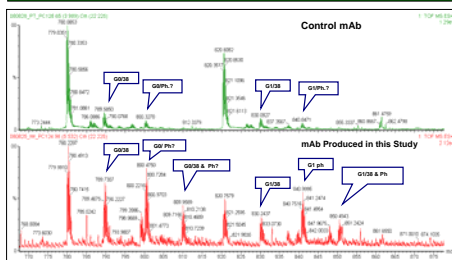


Figure 2. Glycopeptide profile analysis. Denaturated and reduced Protein A purified protein samples were digested with trypsin and acidified with TFA, loaded on a Zorbax 300SB-C18 analytical HPLC column and eluted with a linear gradient of acetonitrile (0.39%/min) at 45°C. The peak corresponding to the glycopeptide was collected, dried, reconstituted in 0.2% formic acid and injected to ESI-MS. The control and the glycopeptides developed in this project had a similar G0/G1/G2 glycan ratio. Other species, notably peaks corresponding to an G0/G1 + 38 Da and those corresponding to phosphorylated forms were also observed in both samples, but were more abundant in the in-house material.

Production Optimization Using CHO CD EfficientFeed™ A and B

CHO CD EfficientFeed™ A and B with different nutritional structures allow accommodating unique cell culture requirements by using them independently and in various combinations. We have applied these combinatorial approaches of CHO CD EfficientFeed™ A and B on recombinant CHO clone # 958 to optimize cell growth and recombinant mAb production in a short period of time (<2 months).

Applying Different Feeding Strategies

During the temperature shift from 37°C to 30°C and induction of recombinant mAb production by cumate, we applied different feeding strategies using CHO CD EfficientFeed™ A and B on the recombinant CHO clone # 958 grown in CD CHO and CD OptiCHO™ media. Different days, with different amount of CHO CD EfficientFeed™ A, B, and combination of A+B in 1:1 ratio were fed into the cell cultures (Table 1).

Table 1. Feeding Protocol

Cnd #	Base Media	CHO CD EfficientFeed	Feed Amount per Day	Feed Days at 30°C
1	CD CHO or CD OptiCHO	A	10%*	1, 3, 5, 7, 9
2	CD CHO or CD OptiCHO	B	10%*	1, 3, 5, 7, 9
3	CD CHO or CD OptiCHO	A + B	10%*	1, 3, 5, 7, 9
4	CD CHO or CD OptiCHO	No Feed	---	---
5	CD CHO or CD OptiCHO	A	15%*	1, 4, 7
6	CD CHO or CD OptiCHO	B	15%*	1, 4, 7
7	CD CHO or CD OptiCHO	A + B	15%*	1, 4, 7
8	CD CHO or CD OptiCHO	No Feed	---	---

% is based on total culture volume

Fed-batch Process Optimization of Recombinant CHO Clone # 958

Recombinant CHO clone # 958 was seeded into duplicate 30 ml cultures at 0.75 x10⁶ cells/ml in the CD CHO and CD OptiCHO™ media without hygromycin. We added cumate ("on" condition) to the batch cultures. After incubation of the cell cultures for 48 hrs at 37°C, 1 µg cumate per one milliliter culture were added to switch the cells to the "on" conditions and then all of cell conditions were transferred to 30°C, 5% CO₂, 110 rpm. Control is without feeding and cumate free ("off" condition). At 30°C, cells growing in CD CHO and CD OptiCHO™ conditions of recombinant CHO clone # 958 were fed with CHO CD EfficientFeed™ A and B in the direction of Feeding Protocol listed in Table 1.

Fed-batch Growth and Viability of Recombinant CHO Clone # 958 Grown in CD CHO Medium

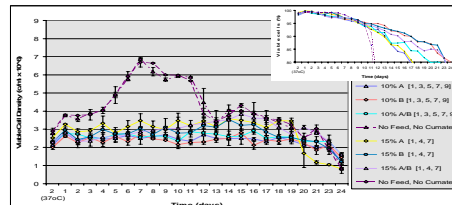


Figure 3. Recombinant CHO clone # 958 was cultured for 54 days prior to setting up the fed-batch experiment. Growth curve was performed two days at 37°C, and rest of time at 30°C using 30 ml cultures in 125 ml shake flasks with starting density of 0.75 x10⁶ cells/ml (5% CO₂, 110 rpm). Samples were withdrawn for cell count at 37°C on day 2 and at 30°C on day 1 through day 24.

Fed-batch Growth and Viability of Recombinant CHO Clone # 958 Grown in CD OptiCHO™ Medium

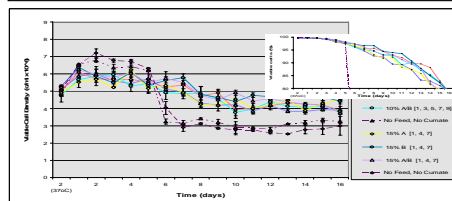


Figure 4. Recombinant CHO clone # 958 was cultured 19 passages prior to growth curve experiment. 30 ml cultures in duplicate in 125 ml shake flasks were seeded at 0.75 x10⁶ cells/ml (5% CO₂, 110 rpm). Samples were withdrawn for cell count at 37°C on day 2 and at 30°C on day 1 through day 16.

Fed-batch Production of Recombinant mAb of CHO Clone # 958 Grown in CD CHO Medium

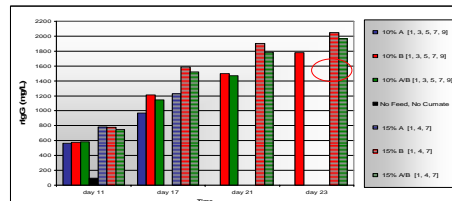


Figure 5. Cells were inoculated at 0.75 x10⁶ cells/ml into a 30 ml working volume in 125 ml shake flask in CD CHO at 37°C for two days (5% CO₂, 110 rpm). On day two 1 µg cumate per one milliliter culture was added and cells were transferred to 30°C, 5% CO₂, 110 rpm. Different fed-batch strategies as illustrated in Table 1. IgG samples were collected on day 11, 17, 21 and 24, until cell viabilities dropped to 80%.

Fed-batch Production of Recombinant mAb of CHO Clone # 958 Grown in CD OptiCHO™ Medium

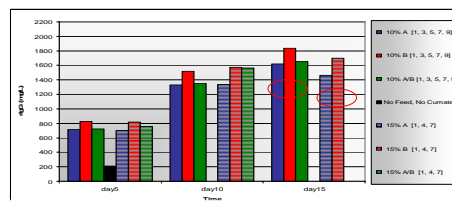


Figure 6. Cultures were seeded in duplicate at 0.75 x10⁶ cells/ml in 125 ml shake flask with 30ml working volume in. CD OptiCHO™ is used as base medium. Cultures were incubated at 37°C, 5% CO₂, and shaken at 110 rpm for two days. On day two 1 µg cumate per one milliliter culture was added and cells were transferred to 30°C, 5% CO₂, 110 rpm. Different fed-batch strategies as illustrated in Table 1. IgG samples were collected until cell viabilities dropped to 80%.

Summary

We have demonstrated that high producing recombinant CHO clones can be developed using the cumate inducible expression system to produce recombinant mAb with comparable glycosylation patterns as commercial standards. The home-made product has molecular masses for HL and LC and isoelectric profiles similar to those of commercial standard (data not shown).

Using chemically defined base media and feeds, we can reach 2 g/liter in very short cell culture development time.