Media and Cell Manipulation Approaches to the Reduction of Adaptation Time in GS NS0 Cell Line

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Abstract: Initial attempts to grow GS NS0 cells in the serum-free media provided by Cambrex failed owing to a lack of certain additions. It was decided to evaluate the addition of cholesterol, cholesterol encapsulated with a cyclodextrin carrier and Glutamine Synthetase Expression Supplements (GSES). Aim was to evaluate if the adaptation time could be reduced, while still maintaining equal to higher viable cell density and IgG antibody production. It was found that GS NS0 cells required the addition of cholesterol to sustain growth in the medium. The addition of cholesterol encapsulated with cyclodextrin with the GSES additives reduced adaptation time to 50 days from 127 days for cholesterol alone.

Key words: GS NS0, CHOLESTEROL, GSES.

1. INTRODUCTION

Serum-supplemented media are normally used to culture mammalian cells, however, growing concerns over serum supply and the potential for exogenous serum contamination has driven the transition to serum-free media. Some cell lines (e.g., cholesterol auxotrophic cell line, NS0) have unique requirements for specific media supplementation to be effective, as demonstrated by Keen and Steward (1). However, the production of purified recombinant proteins has created a demand for media capable of supporting growth without relying on these supplements. The use of serum-free media requires some adaptation effort to ensure the most viable cell population is

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selected for long-term cell culture studies or the continuous production of desired cell derived proteins. Adaptation protocols are tedious, time consuming and vary according to cell lines and production methods. We have demonstrated that with the addition of cholesterol and glutamine synthetase expression media additives (GSEM) adaptation time can be significantly reduced.

2. RESULTS AND DISCUSSION

The GS NS0 cell line in Figure 1, was not adapted to the serum-free media. Initial attempts to get the cell line to grow without the addition of cholesterol failed. When cholesterol was added the media , the NS0 cell line was able to proliferate and sustain growth in the medium. The addition of cholesterol and GSES additives gave an increase in the viable cell density over cholesterol alone. The use of cholesterol encapsulated with cyclodextrin with GSES additives gave the largest increase in viable cell density. The NS0 cells were then adapted using a weaning protocol.

Table 1. Showing the cholesterol requirement of NS0.

Days	Serum-Free Media Only (Cells/mL)	Serum-Free Media + Cholesterol (Cells/mL)	Serum-Free Media + Cholesterol & GSES (Cells/mL)
0	2×10^5	2×10^5	2×10^5
1	1.7×10^5	2.2×10^5	2.2×10^5
2	0.9×10^5	2.9×10^5	3.0×10^5
3	0.3×10^5	3.3×10^5	3.4×10^5
4	0	3.8×10^5	4.2×10^5
5	0	4.3×10^5	4.5×10^5

Mean values plotted. n=3

Table 2. Adaptation time for NS0 cell line.

Compounds Used	Adaptation Time (Days)
Serum-Free Media with Cholesterol	127
Serum-Free Media with Cholesterol and GSES	90
Serum-Free Media with Cholesterol:Cyclodextrin and GSES	50

Table 2, shows the results for adaptation time with the selected components from Table 1. It was observed to take 127 days for the NS0 cell line to adapt to the serum-free media with the addition of cholesterol. Adding GSES additives to the media with cholesterol improved the adaptation time to 90 days for the NS0 cell line. The quickest time observed was 50 days, which included the addition of cholesterol encapsulated with cyclodextrin and GSES additives.

Table 3. Comparison of adapted NS0 serum-free cells in serum-free media, verses NS0 cells in basal media.

Days of Batch	Serum-free Cell Count (Cells/mL)	Basal Media Cell Count (Cells/mL)	Serum-Free IgG Production (µg/mL)	Basal Media IgG Production (µg/mL)
0	2.5×10^5	2.5×10^5		
1	3.4×10^5	3.2×10^5		
2	4.9×10^5	4.6×10^5		
3	7.8×10^5	6.7×10^5	37	35
4	10.7×10^5	8.8×10^5	48	42
5	9.8×10^5	9.1×10^5	62	57
6	7.3×10^5	7.2×10^5	64	55
7	5.4×10^5	5.5×10^5	50	37
8	2.8×10^5	3.1×10^5	32	21
9	1.6×10^5	1.2×10^5		
10	0.9×10^5	0.4×10^5		

Mean values plotted. n=3.

The adapted NS0 cell line in Table 3, was tested to see if the adaptation process had been successful. The serum-free media out-performed the basal media in respect of viable cell density and antibody production. A common observation observed when adapting cell lines to serum-free media is the actual reduction of antibody production in comparison to basal media, even though viable cell density may be proportional to basal media.

3. CONCLUSION

It has been demonstrated in Figure 1, that the NS0 cell line required the addition of cholesterol to maintain growth in the serum-free media used. The addition of GSES additives also aided in maintaining growth. Figure 2 showed that with the additions observed in Figure 1, that the adaptation time can be reduced. To adapt the NS0 cells in the shortest time period it was observed that the additions of cholesterol encapsulated with cyclodextrin and GSES additives gave a 50 day period. The slowest time observed was from only adding cholesterol alone leading to a 127 day period. Subsequent performance testing, Figure 3, showed that the NS0 cell line had a higher viable cell density and equal to higher IgG productivity in comparison to basal media.

REFERENCES

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