

Corning® hybrigro™ SF Medium: The Choice for Hybridoma Cell Growth and Protein Production

Application Note

CORNING

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Introduction

The wide application of monoclonal antibodies (mAbs), such as targeted therapy for cancer, disease diagnosis, and disease prevention, has been greatly enhanced by the development of hybridoma technology by Köhler and Milstein in the 1970s¹. As hybridoma cells are the fusion of antibody producing B-lymphocytes and myeloma cells, they are very sensitive to culture. As the demand for mAbs for use in clinical applications increases, the ability to culture hybridoma cells, especially in serum-free conditions, is becoming vital.

Corning hybrigro SF medium is a complete, animal-free, defined medium, and is specifically developed for serum-free growth and production of antibodies. This medium has been shown to enable higher protein production compared to serum-containing medium².

Hybridoma cells can be cultivated in either shaking mode or static mode to meet researchers' or manufacturers' needs. In this study, we compared Corning hybrigro SF medium with Competitor products to evaluate their hybridoma cell proliferation and protein production.

Materials and Methods

Shaking mode culture of AE-1 cells

30 mL of 1×10^5 cells/mL of AE-1 cells were seeded into 125 mL Erlenmeyer flasks (Corning Cat. No. 431405) containing either Corning hybrigro SF medium (Corning Cat. No. 40-215-CV), Competitor medium, or the control medium of DMEM (Corning Cat. No. 10-013-CV) +10% FBS (Corning Cat. No. 35-079-CV). The cells were then incubated for 4 days at 5% CO₂ and 37°C at 90 rpm in a shaking incubator. Cell density and viability were assessed by Trypan blue exclusion method daily.

Static mode culture of IL21.23 cells

5 mL of 1×10^5 cells/mL of IL21.23 cells were seeded into T-25 flasks (Corning Cat. No. 353108) containing either Corning hybrigro SF medium, Competitor medium, or DMEM+10% FBS medium. The cells were then incubated for 4 days at 5% CO₂ and 37°C. Cell density and viability were assessed by Trypan blue exclusion method after whole culture period.

Protein production assay

1 mL of cell suspension was collected in a 15 mL tube, and supernatant was obtained by spinning down the tube at 800 xg for 5 min. Protein IgG assay was conducted by using Easy-Titer™ Assay Kits (Thermo Fisher Cat. No. 23300) according to the manufacturer's protocol.

Results and Discussion

AE-1 cell growth and protein production

Using the shaking incubator, AE-1 cells cultured in Corning hybrigro SF medium showed the highest cell density after culturing for 4 days, which was 4 times higher than cells in Competitor media and the control medium (DMEM+10% FBS), as shown in Figure 1.

The mouse protein IgG produced by AE-1 cells cultured in Corning hybrigro SF medium also outperformed the Competitor media and the control medium, resulting in 25 µg protein amount in total, as shown in Figure 2.

Figure 3, combining cell number and protein production, illustrates that AE-1 cell cultured in different conditions showed very different protein production ability. Protein produced in Corning hybrigro SF medium and Competitor H medium can eventually produce 12 pg per AE-1 cell. Competitor GC, GS, and GP all have lower protein production ability for AE-1 cells.

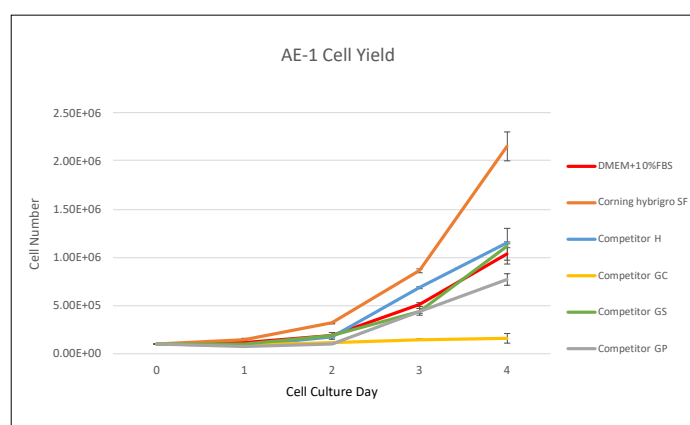


Figure 1. AE-1 cell proliferation density curve for 4 days culture in Corning hybrigro SF medium, Competitor media and control medium (DMEM+10% FBS).

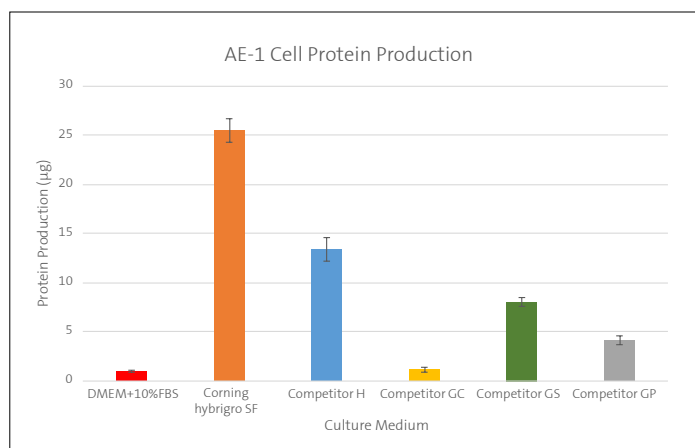


Figure 2. AE-1 cell protein production in Corning hybrigro SF medium, Competitor media and control medium (DMEM+10% FBS).

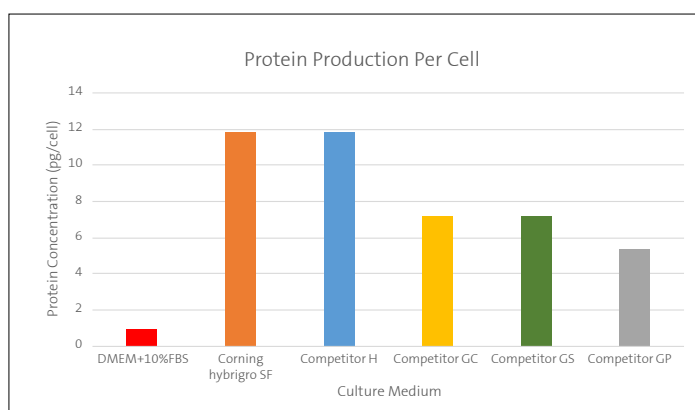


Figure 3. Mouse IgG protein production per AE-1 cell in Corning hybrigro SF medium, Competitor media and control medium (DMEM+10% FBS).

Hybridoma IL21.23 cell growth and protein production

In static mode of culture, IL21.23 cell cultured in Corning® hybrigro™ SF medium had comparable expansion ability as Competitor H, while they both showed much higher cell growth than Competitor GD, GS, GP, and control medium, as shown in Figure 4.

A similar trend of mouse protein IgG amount was found in Corning hybrigro SF medium and Competitor H. The expected protein amount reached 14 to 16 µg. However, relatively very little protein IgG were found in Competitor GD, GS, GP, and the control medium, as shown in Figure 5.

Figure 6 shows mouse IgG protein production per IL21.23 cell in Corning hybrigro SF medium, Competitor media and control medium (DMEM+10% FBS). Nearly 5 to 6 pg of protein was produced from both Corning hybrigro SF medium and Competitor H medium, and relatively lower protein was found in other Competitor media.

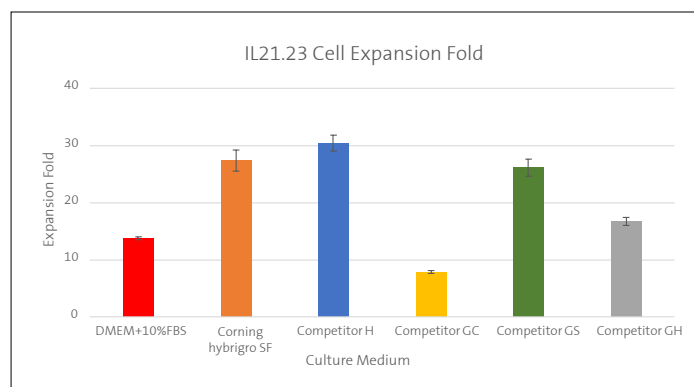


Figure 4. IL21.23 cell expansion fold after 4 days culture in Corning hybrigro SF medium, Competitor media and control medium (DMEM+10% FBS).

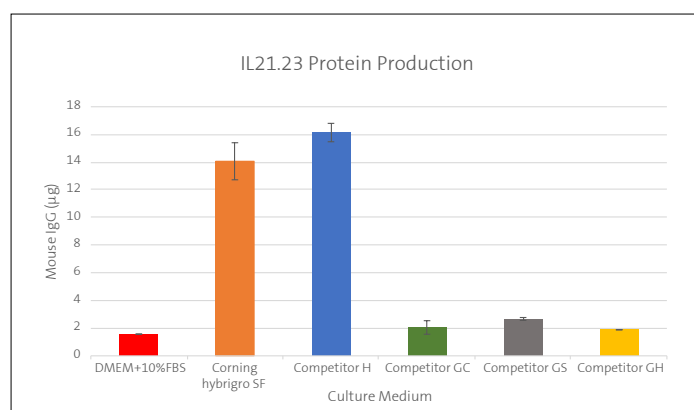


Figure 5. IL21.23 cell protein production in Corning hybrigro SF medium, Competitor media and control medium (DMEM+10% FBS).

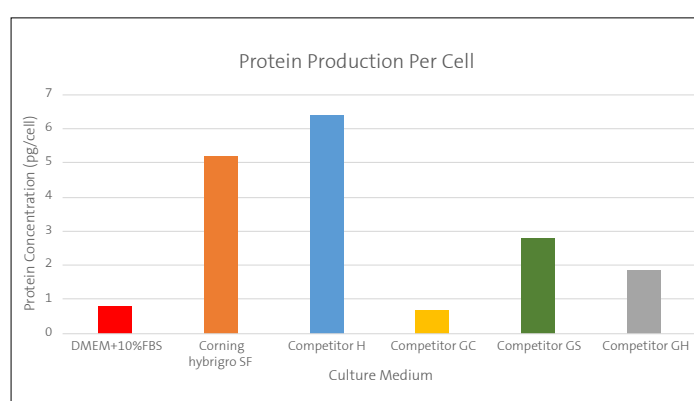


Figure 6. Mouse IgG protein production per IL21.23 cell in Corning hybrigro SF medium, Competitor media and control medium (DMEM+10% FBS).

Conclusions

- ▶ Corning® hybrigro™ SF medium can support both shaking culture and static culture of hybridoma cell growth and protein production.
- ▶ AE-1 cell and IL21.23 cell both showed outstanding cell proliferation and protein production ability cultured in Corning hybrigro SF medium.
- ▶ Corning hybrigro SF medium performed better in cell growth and protein production than corresponding Competitor products.

References

1. Bretton PR, Melamed, MR, Fair WR, Cote RJ (1994). Detection of occult micrometastases in the bone marrow of patients with prostate carcinoma. Prostate. 25(2):108-14.
2. Hilary Sherman and Mark E Rothenberg. Corning hybrigro SF medium for high density hybridoma culture and increased production (2014) [Corning Lit. Code CLS-CG-AN-004].

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