Corning® Iscove's Modification of Dulbecco's Medium

An Ideal Choice for Rapid Proliferation of Cultures

Technical Report

CORNING

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Introduction

Cultured cells require several components for proper growth and health. Growth medium is one of these critical components, that not only supports viability, but also enables the cells to to behave in an *in vivo*-like manner. Using media that is not optimized for the cells being cultured may have drastic implications on cell health, doubling time, and functionality. It supports cell growth by both maintaining the culture pH and by providing essential nutrients. The goal of this study was to evaluate Corning Iscove's Modification of Dulbecco's Medium (IMDM) compared to other commercially available IMDM with the same formulation. IMDM is a rich medium optimized for culturing cells at high densities, thereby making it an ideal choice for the growth and maintenance of many cell types. Equivalent performance is demonstrated by analyzing the growth characteristics of two different cell lines, HEK-293 and 5/9m alpha3-18 (a CHO-K1 derivative), cultured in Corning IMDM compared to three competitor media manufacturers.

IMDM Assessment with HEK-293

To compare the different media types, HEK-293 cells were chosen because these cells are commonly used as a mammalian expression system. HEK-293 (ATCC Cat. No. CRL-1573) cells were thawed and counted with the BioProfile® Flex analyzer (Nova Biomedical). Cells were reconstituted in either Corning IMDM (Cat. No. 10-016-CM) supplemented with 10% fetal bovine serum (FBS) (Corning Cat. No. 35-010-CV), or one of three competitor IMDM containing 10% FBS. HEK-293 cells were seeded onto Corning® T-25 flasks with CellBIND® surface (Corning Cat. No. 3289) at a concentration of 5,000 cells/cm^2. Cells were cultured for four days in a humidified incubator at 5% CO_2 and 37°C. After four days, representative photomicrographs were taken using the Evos® FL microscope (Life Technologies). The cells were then harvested with trypsin (Corning Cat. No. 25-052-CV) and counted on the Vi-CELL® (Beckman Coulter) cell counter. HEK-293 cells appeared to have similar morphology and confluence (Figure 1) independent of which IMDM was used for propogation.

Upon harvesting the cells, no statistically significant difference in cell density was detected when comparing the growth rates in the different media (Figure 2).

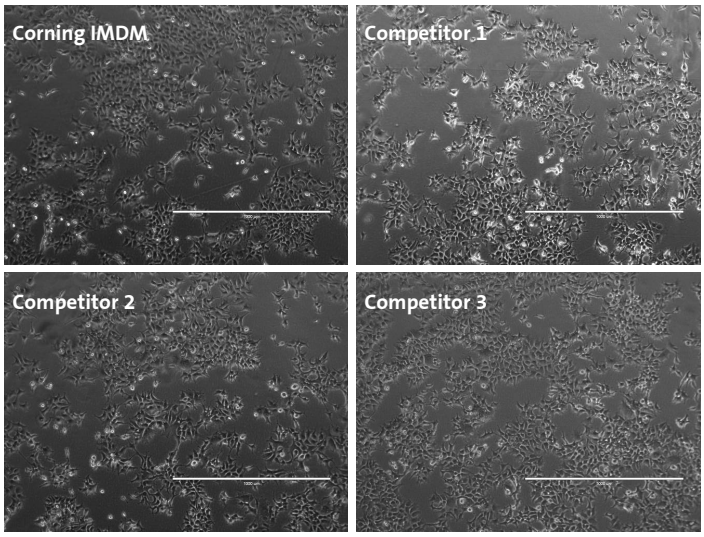

Figure 1. Similar cell confluence and morphology of HEK-293 in various IMDM at 40X magnification.

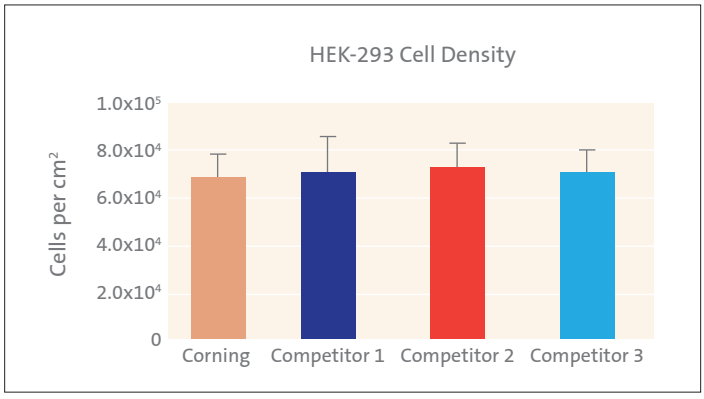

Figure 2. No statistical difference in HEK-293 cell densities after four days of culture (ANOVA – Newman-Keuls Post-Test).

IMDM Assessment, 5/9 m alpha3-18

To assess the functional impact of the different media on cellular physiology, 5/9 m alpha3-18 were chosen for their ability to secrete macrophage colony stimulating factor (M-CSF). 5/9 m alpha3-18 (ATCC Cat. No. CRL-10154) cells were thawed and counted with the BioProfile® Flex analyzer and reconstituted in either Corning® IMDM supplemented with 10% FBS or one of the three competitor IMDM containing 10% FBS. 5/9 m alpha3-18 cells were seeded onto Corning CellBIND® surface 6-well cell culture plates (Corning Cat. No. 3335) at a concentration of 10,000 cells/cm^2. Cells were cultured for four days in a humidified incubator at 5% CO_2 and 37°C. After four days, representative photomicrographs were taken using the Evos® FL microscope. An additional 400 µL of media was collected and frozen for later quantification of macrophage colony stimulating factor (M-CSF). Finally, cells were harvested with trypsin and counted on the Vi-CELL® cell counter. M-CSF production was assessed by following the protocol provided for the Human M-CSF Immunoassay (R&D Systems Cat. No. DMC00B). 5/9 m alpha3-18 cells exhibited similar morphology and equivalent cell densities, regardless of which IMDM was used for the cell culture (Figures 3 and 4). Lastly, an M-CSF immunoassay was conducted to assess the amount of M-CSF produced by 5/9m alpha cells at day 4 in culture with each IMDM. The amount of M-CSF produced per cell was determined to be similar, regardless of which medium was tested (Figure 5).

Conclusions

- Corning IMDM is a highly enriched synthetic medium designed for rapid proliferation of cultures.
- Equal cell densities can be achieved with Corning IMDM as compared to other commercially available IMDM when culturing HEK-293 or 5/9 m alpha3-18 cells.
- 5/9 m alpha3-18 cells produce equivalent M-CSF when propagated in Corning IMDM or other commercially available IMDM.

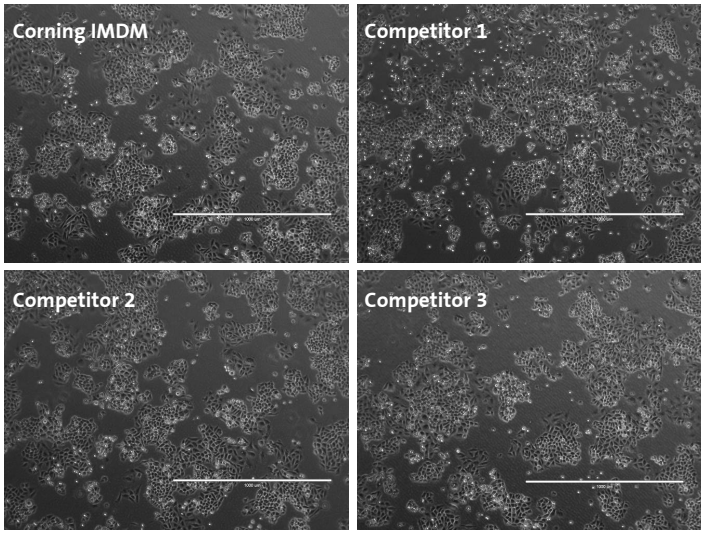

Figure 3. Similar cell confluence and morphology of 5/9 m alpha3-18 cells in various IMDM at 40X magnification.

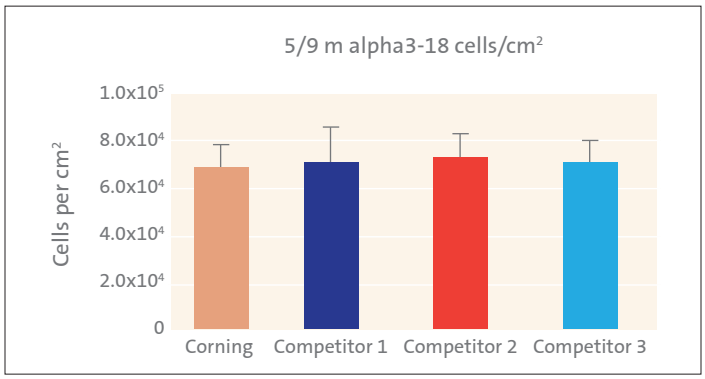

Figure 4. No statistical difference in cell densities after four days of culture (ANOVA – Newman-Keuls Post-Test).

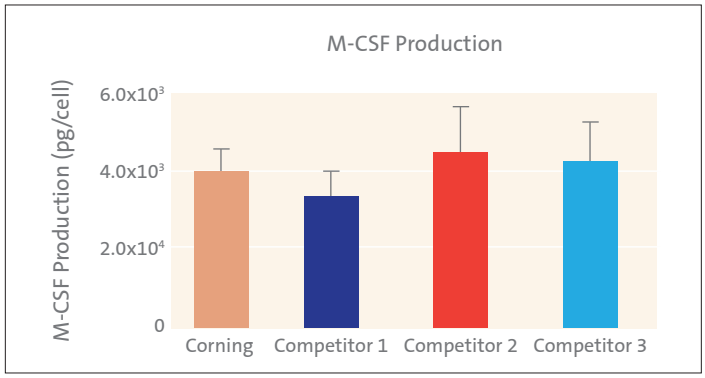

Figure 5. No statistical difference in M-CSF production after four days of culture (ANOVA – Newman-Keuls Post-Test).

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