

Assessing the Differences in Fresh, Commercially-Available Hybridoma Media

Repligen Corp. now owns the life sciences PAT product portfolio of 908 Devices Inc. Please contact Repligen for further inquiries.

Background

Hybridomas are hybrid cell lines used to produce monoclonal antibodies. They are made by fusing B cells with myelomas to make immortalized and productive cell lines. Early in the field of hybridoma development, researchers used base media with serum supplementation to support the growth and viability of the cell lines. An issue with serum use was the lot-to-lot variability associated with using this undefined component. As the desire to minimize run-to-run variability emerged, the focus shifted to culturing hybridomas in chemically-defined media. A common approach was to mix base media like DMEM/F12 (Dulbecco's Modified Eagle Medium (DMEM) and Ham's F-12 nutrient mixture) with recombinant supplements. Lower antibody production was a drawback encountered using this approach. To increase output, teams formulated chemically-defined media specifically for hybridoma manufacturing with higher levels of some media components (e.g., amino acids) compared to DMEM/F12. The higher levels of the media components supported better hybridoma growth and productivities, but the exact formulations were proprietary to the vendors.



The Experiment

Two different chemically-defined cell media intended for hybridoma culturing were tested. Both media samples were handled following the manufacturers' instructions. The samples were diluted 50X before analysis on the PATsmart™ REBEL® System with no additional sample preparation. Each vendor's medium was run five times, and levels were compared to the known formulation values of DMEM/F12 components.

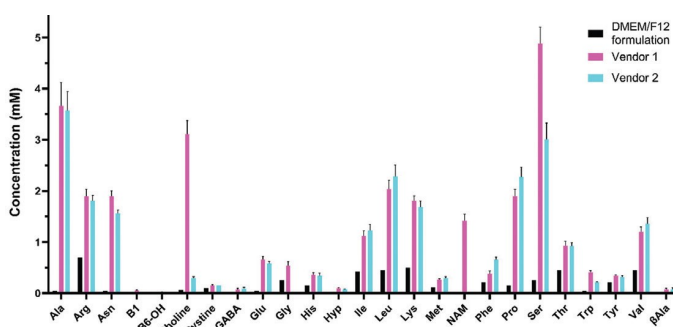


Figure 1. The concentrations of the amino acids, vitamins, and other nutrients in chemically-defined hybridoma media from two vendors compared to the formulation values of DMEM/F12. Error bars are from the standard deviation of n = 5 replicates.

Discussion

The concentrations of media components are essential to consider when doing media optimization studies. Here, the nutrient levels in two chemically-defined media were significantly higher than in the DMEM/F12 base media, which is typically used with serum supplementation. The levels of the amino acids alanine (Ala; 73X and 71X higher), asparagine (Asn; 38X and 31X higher), glutamic acid (Glu; 13X and 11X higher), proline (Pro; 13X and 15X higher), and serine (Ser; 20X and 12X higher) were significantly higher than the base media formulation levels for Vendors 1 and 2, respectively. All amino acids were at least 50% higher in the chemically-defined media compared to the base media except for glycine (Gly), which was not detected in Vendor 2's media. Other media components like choline were 49X and 5X higher than the base media for Vendor 1 and 2, respectively. Vitamins (B1, B6-OH, NAM) were part of Vendor 1's medium and were at least double the levels of DMEM/F12. When transitioning from a general base media to a chemically-defined media, run a quick media analysis on the REBEL Analyzer to give you the insight to help boost hybridoma growth and productivity.