

Hybri-CYTE™ Cell Growth Supplement

An FBS Replacement for Hybridoma Culture

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Abstract

Traditional production systems rely on the use of large quantities of animal sera such as Fetal Bovine Serum (FBS) to produce therapeutics, diagnostic reagents and other biologics. This reliance on FBS is undesirable due to the cost fluctuation, possible source for contamination, lot-to-lot variability, and complicated downstream processing. Advances in cell culture media development have allowed mammalian culture systems to gravitate towards a serum-free environment. Hybri-CYTE supplement, a newly formulated product from Millipore, is a serum-free cell culture supplement that completely replaces the use of FBS in hybridoma culture. Hybri-CYTE supplement supports robust growth and viability of murine hybridoma cells while promoting comparable or increased monoclonal antibody production compared to media containing 10% FBS. Hybri-CYTE supplement is an effective and consistent alternative to FBS that uses high quality components thus reducing the concerns of quality and performance variation typically associated with FBS.

Introduction

It is accepted that FBS contains multiple beneficial components, including growth factors, cytokines and hormones that promote cell growth. However, FBS has numerous disadvantages including cost fluctuation, lot-to-lot variability, and complications in down stream protein purification and processing. Because of these disadvantages, FBS replacements capable of promoting cell proliferation and protein expression would be technically and economically beneficial for research and industry bioprocessing.

Hybri-CYTE supplement, a new cell culture product from Millipore, was developed as an effective and consistent FBS replacement to support murine hybridoma proliferation and protein expression. This supplement provides lot-to-lot consistency thereby eliminating the need for extensive lot screening typically required for FBS. Hybri-CYTE supplement is also easy to use and requires no adaptation of culture from FBS-containing environment. In our hybridoma culture testing, Hybri-CYTE supplement has demonstrated its utility and performance by matching or exceeding 10% FBS for growth promotion, cell viability, and protein production in plates, T-flasks, and spinner flasks with numerous murine hybridomas.

Materials and Methods

Cell Lines

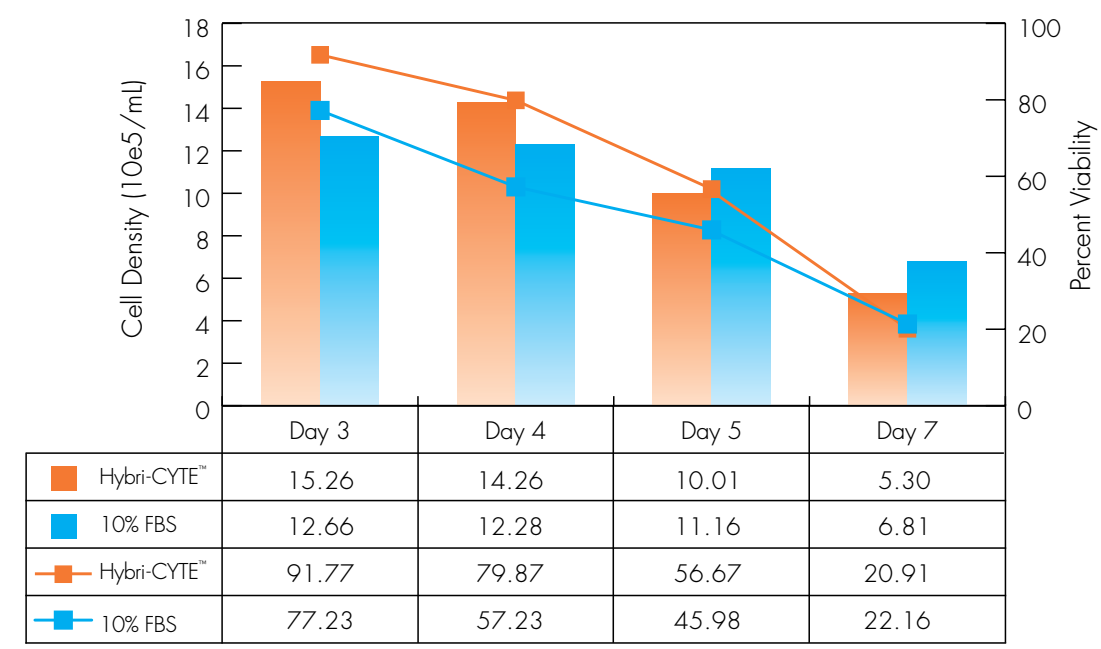
In the experiments demonstrated here, MK2.7 (ATCC # CRL-1909) and a proprietary hybridoma cell line denoted as "Cell Line D" were tested using Hybri-CYTE supplement while media containing 10% FBS served as the positive control.

Plate Studies

To begin the plate studies, actively growing log phase cells were harvested, washed and seeded into plates at 1×10^5 cells per well in DMEM containing 10% FBS as positive control and Hybri-CYTE supplement (50mL/L media) as the test condition. For all plate experiments, 24-well non-tissue culture treated plates were used. Growth and viability were measured by propidium-iodide uptake using a FACS growth promotion assay established at Millipore. Total IgG production was measured using an ELISA assay (Bethyl Labs) specific for the different IgGs being expressed by each of the cell lines.

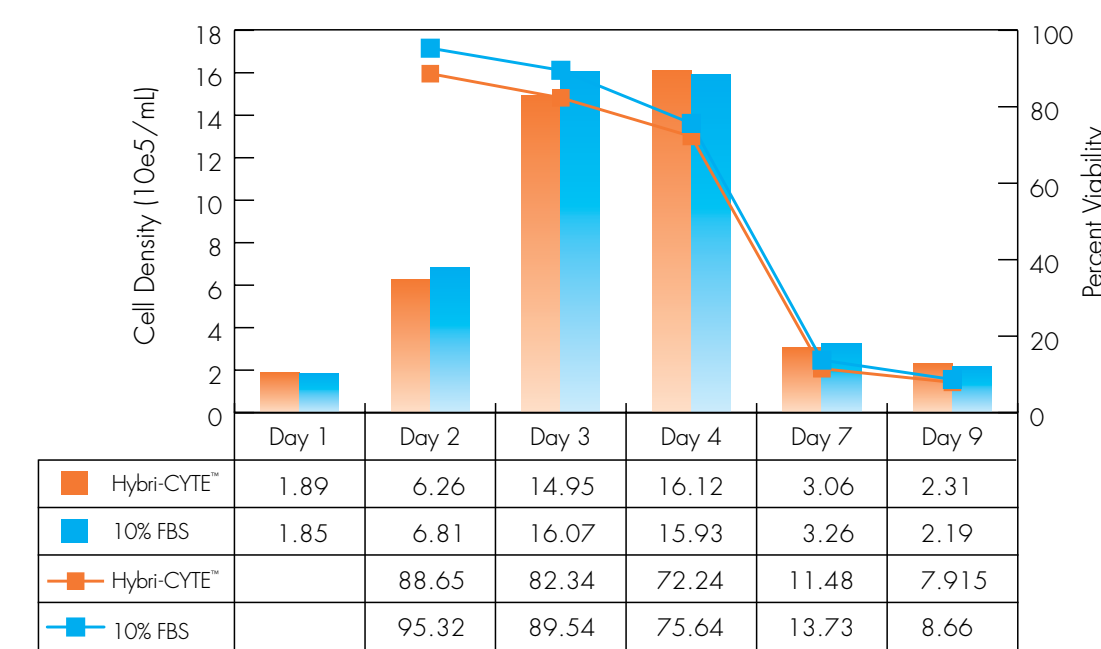
Results

Figure 1. MK2.7 Cell Growth Comparison



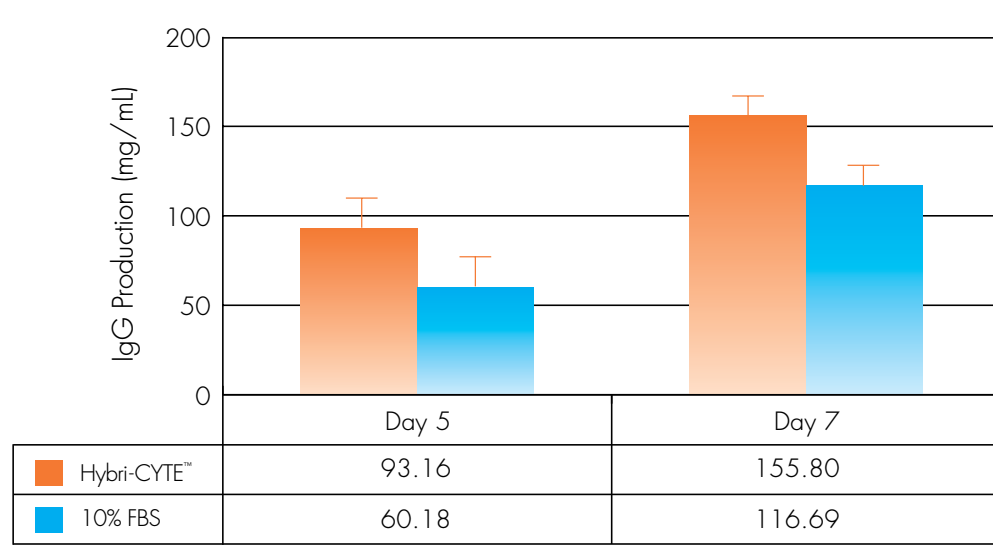
Cell density (bars) and viability (lines) of MK2.7 culture grown in media containing Hybri-CYTE supplement compared to culture grown in media containing 10% FBS. Comparable cell density and higher viability were shown in culture in Hybri-CYTE supplement.

Figure 3. Cell Line D Cell Growth Comparison



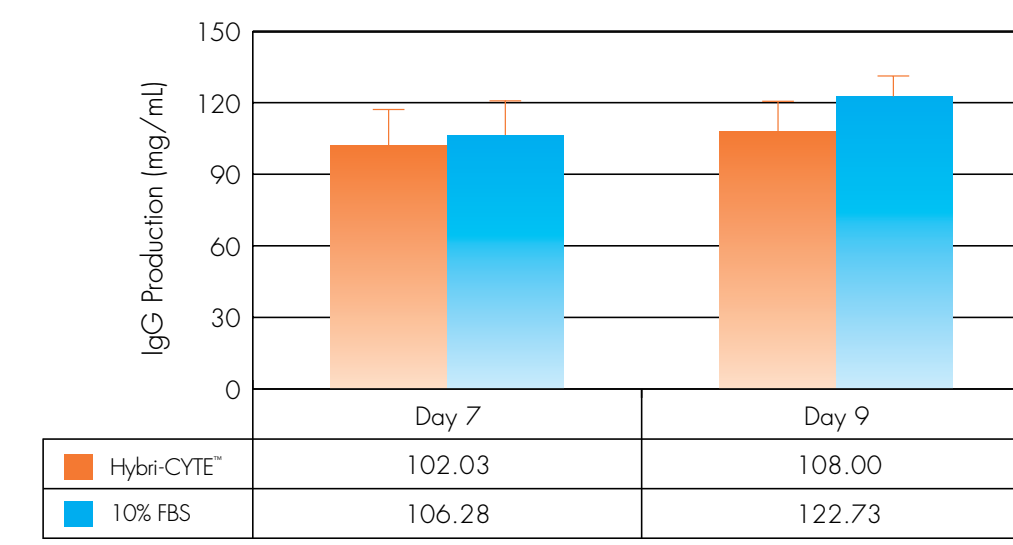
Cell density (bars) and viability (lines) of proprietary Cell Line D culture grown in media containing Hybri-CYTE supplement compared to culture grown in media containing 10% FBS. Comparable cell density and viability were observed in the two conditions.

Figure 2. MK2.7 Protein Production Comparison



Cumulative IgG production in MK2.7 culture grown in media containing Hybri-CYTE supplement compared to media containing 10% FBS. Over 30% higher IgG production on day 7 was observed in Hybri-CYTE.

Figure 4. Cell Line D Protein Production Comparison



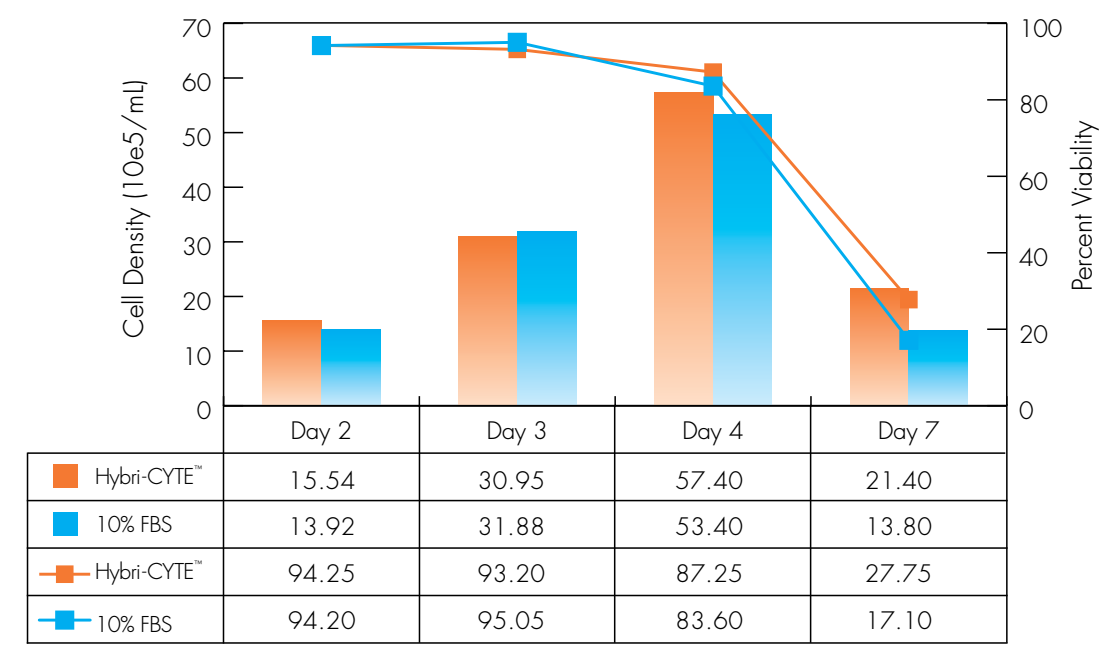
Cumulative IgG production in proprietary Cell Line D culture grown in media containing Hybri-CYTE supplement was comparable to IgG production in media containing 10% FBS.

Spinner Flask Studies

To begin the spinner flask studies, actively growing log phase cells were harvested, washed and seeded at density of either 2.5×10^5 or 6×10^5 cells per mL in either 10% FBS as positive control or Hybri-CYTE supplement (50 mL/L media) as the test condition in DMEM. All spinner cultures utilized 250 mL spinner flasks were set to stir at ~45 rpm. Cell density and viability were measured by trypan blue incorporation. Total IgG production was measured using an ELISA assay (Bethyl Labs) specific for the different IgGs being expressed by each of the cell lines.

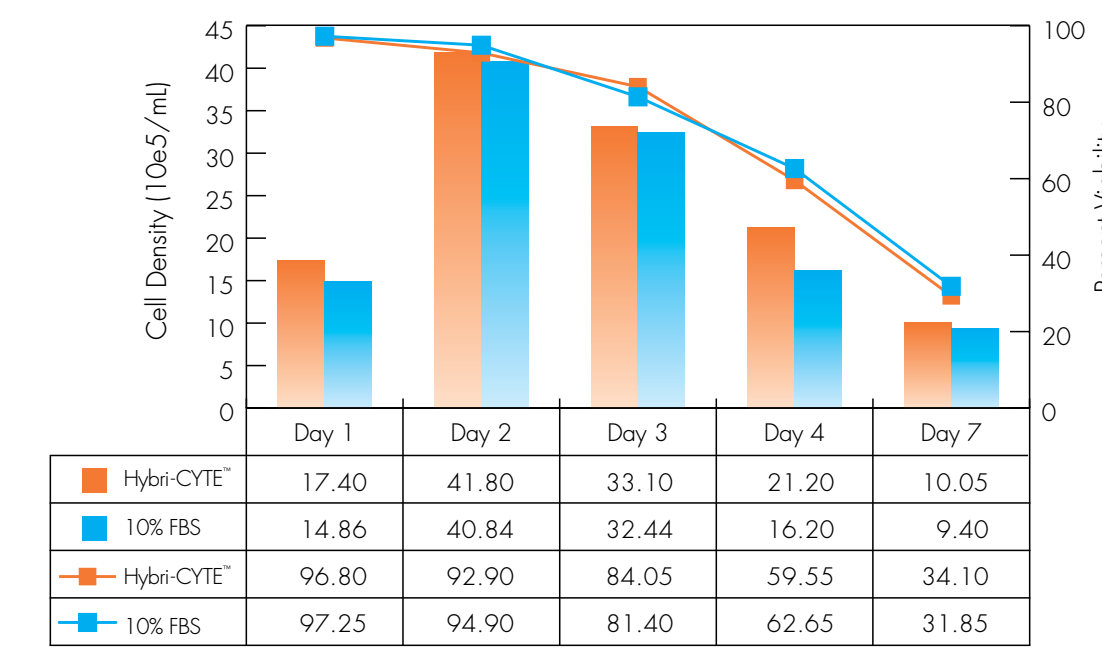
Results

Figure 5. MK2.7 Cell Growth Comparison



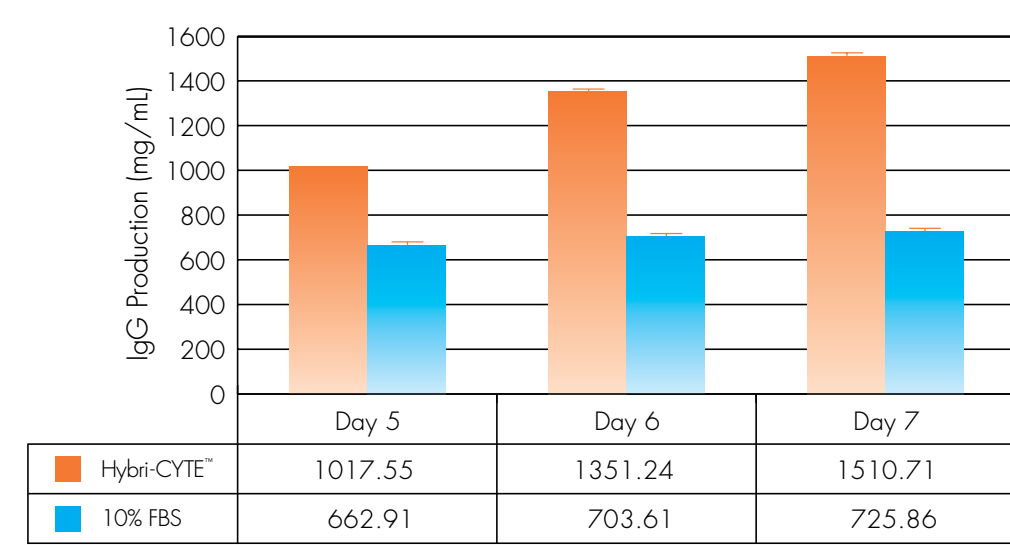
Comparable cell density (bars) and viability (lines) of MK2.7 culture were achieved in spinner flasks in media containing Hybri-CYTE supplement and in media containing 10% FBS.

Figure 7. Cell Line D Cell Growth Comparison



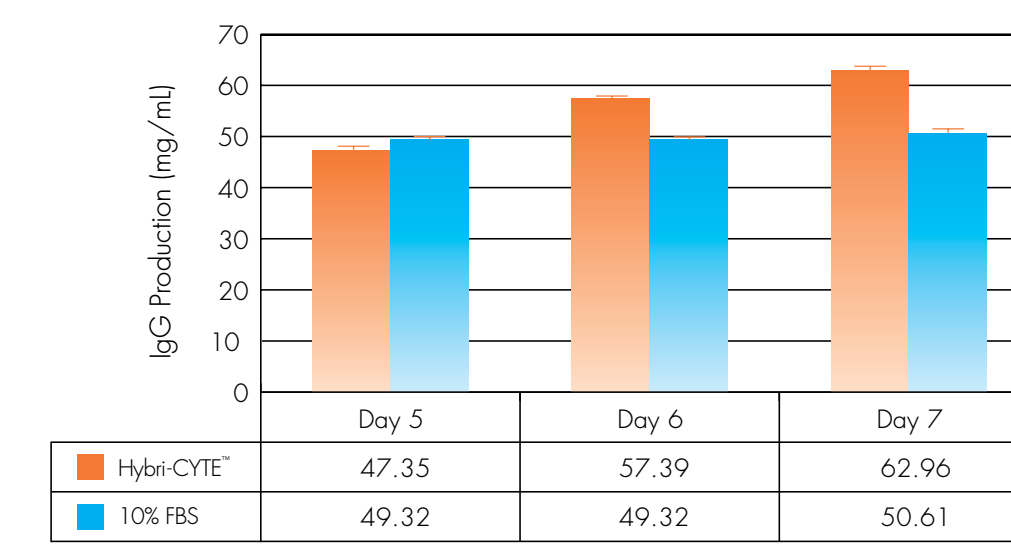
Comparable cell density (bars) and viability (lines) of proprietary Cell Line D culture were achieved in spinner flasks in media containing Hybri-CYTE supplement and in media containing 10% FBS.

Figure 6. MK2.7 Protein Production Comparison



Cumulative IgG production in MK2.7 culture grown in spinner flasks in media containing Hybri-CYTE supplement compared to media containing 10% FBS. Hybri-CYTE supplement provided twice the amount of IgG production on day 7 compared to FBS.

Figure 8. Cell Line D Protein Production Comparison



Cumulative IgG production in MK2.7 culture grown in spinner flasks in media containing Hybri-CYTE supplement compared to media containing 10% FBS. Hybri-CYTE supplement provided 24% higher amount of IgG production on day 7 compared to FBS.

Conclusions

Hybri-CYTE supplement is a serum-free supplement developed to replace the use of FBS in hybridoma culture. As shown in our experiments, Hybri-CYTE supplement provides the following features and advantages:

1. Comparable performance to FBS – Hybri-CYTE supplement exhibits equal or greater performance in cell growth and antibody production to FBS in plates, Tflasks, and spinner flask formats.
2. Ease of use. Only one mixing step with basal media and glutamine of choice is required to give you a serum-free media to start your hybridoma culture.
3. No adaptation required – All cell lines tested could be transferred directly from medium containing 10% FBS into media containing Hybri-CYTE supplement. Consistent growth rate and antibody production was maintained in subsequent subcultures in Hybri-CYTE supplement.
4. Lot-to-lot consistency – Hybri-CYTE supplement is a serum-free supplement formulated with controlled raw materials with fully traceable sources. This allows for consistent performance from batch-to-batch, eliminating lot qualification typically required for FBS.
5. Easy to handle and store.

More Information

Customer Service

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