Thermo Scientific
HyClone FetalClone I, II and III:
Serum Alternatives to FBS

Bobbie Thompson and Nathan Allen, Thermo Fisher Scientific

Key Words
Thermo Scientific™ HyClone™ Fetal Bovine Serum, FBS Alternatives, Thermo Scientific™ HyClone™ FetalClone™, Bovine Calf Serum, Cell Culture, Serum Supplementation, CHO-K1, MRC-5, NS0, BHK-21, Vero, AIF, Hybridoma, Myeloma

Goal
The Thermo Scientific HyClone FetalClone line of HyClone Fetal Bovine Serum (FBS) alternatives were compared to FBS and Iron-Supplemented bovine calf serum on 6 cell types. Data demonstrates that these alternatives match or outperform FBS in cell culture growth promotion in many applications. As these products are made from raw materials more readily available than FBS, they are recommended as cost effective alternatives to standard FBS.

Introduction
Anglers and surfers have it easy compared to those of us doing cell culture. The oceans are abundant and the tides are predictable, but the supply of fetal bovine serum (FBS) is neither presently abundant nor predictable. It ebbs and flows with the uncertainties of politics, animal husbandry, weather, and the beef packing industry.

Despite varying quality and sometimes short supply, researchers have depended for decades on FBS to maintain their cell cultures because it works well. Fortunately for those of us practicing today, there are plentiful, consistent, and economical substitutes for FBS.

We have taken the lead in creating a number of FBS alternatives using readily available raw materials to decrease the cost of cell culture supplementation and improve cell culture yields. The Thermo Scientific HyClone FetalClone line of products are specially designed FBS Alternatives that provide for the nutritional needs of a culture while keeping total cost of cell culture down. These products often outperform FBS in growth promotion.

In this study, we compared our three FetalClone products to FBS and iron-supplemented Bovine Calf Serum (BCS) for their ability to promote cell culture growth.
The HyClone FetalClone product family

FBS is commonly used as a supplement in cell culture because it is very nutrient-rich and contains a host of compounds and growth factors that promote healthy culture conditions. One of the drawbacks of using FBS is its variable nature. Levels of key nutrients vary widely between lots. BCS, a commonly used alternative to FBS because of its lower price and greater supply, is more uniform batch-to-batch than FBS. Its drawback, however, is that it is lower than FBS in certain nutritional factors. With decades of experience, our scientists developed the HyClone FetalClone line of products to leverage the strengths of both FBS and BCS. HyClone FetalClones I, II, and III are composed of specially processed BCS and supplements. By supplementing the low nutrients in calf serum, Thermo Scientific brings concentrations of the critical nutrients in our HyClone FetalClone products up to those found in FBS.

What You Need To Know

Each HyClone FetalClone is optimized for the growth of different cell types (see accompanying chart).

<table>
<thead>
<tr>
<th>HyClone FetalClone</th>
<th>Principle use</th>
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<tbody>
<tr>
<td>HyClone FetalClone I</td>
<td>Hybridomas</td>
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<tr>
<td>HyClone FetalClone II</td>
<td>CHO cells</td>
</tr>
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<td>HyClone FetalClone III</td>
<td>Broad application base, including fibroblasts</td>
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<thead>
<tr>
<th></th>
<th>IgG (mg/mL)</th>
<th>Total Protein (g/dL)</th>
<th>Iron (μg/dL)</th>
<th>Transferrin (mg/dL)</th>
<th>Cholesterol (mg %)</th>
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<tbody>
<tr>
<td>HyClone Fetal Bovine Serum</td>
<td>0.2</td>
<td>3.9</td>
<td>175</td>
<td>188</td>
<td>35</td>
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<tr>
<td>HyClone FetalClone I</td>
<td>0.1</td>
<td>4.1</td>
<td>558</td>
<td>556</td>
<td>101</td>
</tr>
<tr>
<td>HyClone FetalClone II</td>
<td>0.1</td>
<td>4.1</td>
<td>557</td>
<td>559</td>
<td>111</td>
</tr>
<tr>
<td>HyClone FetalClone III</td>
<td>0.1</td>
<td>3.6</td>
<td>558</td>
<td>480</td>
<td>71</td>
</tr>
<tr>
<td>Iron Supplemented Calf Serum</td>
<td>14</td>
<td>7.0</td>
<td>630</td>
<td>647</td>
<td>118</td>
</tr>
</tbody>
</table>

Table 1. Critical component levels in FBS and in four FBS substitutes.

Methods and Materials

We investigated cell culture yields of six different cell lines, using media containing serum or serum alternatives as described below. All yields are normalized to those obtained with FBS.

Cell lines tested:
- MRC-5 (human lung fibroblasts, ATCC CCL-171)
- BHK-21 (Syrian golden hamster kidney fibroblasts, ATCC CCL-10)
- Vero (African green monkey kidney, ATCC CCL-81)
- CHO-K1 (Chinese hamster ovary, ATCC CCL-61)
- AIF (alpha intermediate filament hybridoma, ATCC TIB131)
- NS0 (myeloma, ECACC #85110503)

Sera and FBS Alternatives tested:
- FBS (control)
- FetalClone I (optimized for hybridomas)
- FetalClone II (optimized for CHO-K1 cells and derivative cell lines)
- FetalClone III (optimized for fibroblasts)
- Iron-Supplemented Calf Serum
All cultures were grown in T-25 cell culture flasks using 10 mL of appropriate media supplemented with 10% serum. All conditions (except control FBS) were conducted on three lots of sera to test lot-to-lot consistency. Control FBS is a product of pooled-lots of FBS. We seeded flasks at ATCC recommended cell concentrations, incubated them at 37°C, and checked daily for confluency. When any condition reached 100% confluency, all conditions were trypsinized and counted. We normalized performance to the FBS control. The results are presented as relative yields in Figures 1-6. Conditions that produce more cells than the FBS control have values greater than 1.0 (Table 2).

<table>
<thead>
<tr>
<th>Table 2. Cell yields normalized to FBS control. Numbers greater than one indicate that the serum (in the left column) outperforms FBS with that cell line (in the top row).</th>
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<tbody>
<tr>
<td>Control FBS</td>
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<tr>
<td>HyClone FetalClone I</td>
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<tr>
<td>HyClone FetalClone II</td>
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<tr>
<td>HyClone FetalClone III</td>
</tr>
<tr>
<td>Iron-Supplemented Calf Serum</td>
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</table>

![Figure 1. Relative yields of MRC-5 cells cultured in Dulbecco’s Modified Eagle’s (DME)/high glucose medium supplemented with 10% serum. HyClone FetalClone III outperforms FBS in this application.](image)

**Results and Discussion**

The HyClone FetalClone family of products are proprietary serum products. They are composed of specially processed bovine calf serum and supplements that enhance performance with specific cell types. A characteristic of all the HyClone FetalClones is very low immunoglobulin content in comparison to calf serum, and high iron, transferrin, and cholesterol levels in comparison to FBS (Table 1). Based on the results presented here, the HyClone FetalClone family is an effective alternative to FB for many different cell types.

We recommend HyClone FetalClone I as an alternative to FBS for culturing myeloma and hybridoma cell lines. Low immunoglobulin levels, lower cost, and excellent growth of myeloma and hybridoma cells (Table 2 and Figures 5 and 6) are demonstrated advantages of this serum alternative over FBS.

HyClone FetalClone II was developed and is recommended for CHO derived cells lines and produces the highest CHO cell yields, more than 50% greater than FBS (Table 2 and Figure 4).

HyClone FetalClone II also produces the highest yielding NS0 cultures, about 25% higher than FBS (Table 2 and Figure 6). For the other four cell lines, the performance of HyClone FetalClone II was comparable to FBS (Table 2). FetalClone II was the best performing serum on BHK-21 cells (Figure 3).

HyClone FetalClone III, developed for fibroblasts and as a more general use FBS alternative produced the highest yields for MRC-5 cells. It also produced relatively high yields compared to FBS in VERO, CHO and NS0 cultures (Table 2 and Figures 1, 2, 4 and 6).

In contrast to the HyClone FetalClone products, Iron-Supplemented Calf Serum yields are generally the same as, or a bit less than, FBS yields. NS0 myeloma and hybridoma lines are exceptions, having greater yields in Iron-Supplemented BCS than in FBS (Table 2). Despite the greater yields, higher immunoglobulin levels relative to FBS (Table 1) often preclude the use of Iron-Supplemented BCS for monoclonal antibody production.
Figure 2. Relative yields of VERO cells cultured in Minimum Essential Medium (MEM) supplemented with 10% serum. HyClone FetalClone II, FetalClone III, and Iron Supplemented Calf Serum are all good alternatives to FBS for VERO cells.

Figure 3. Relative yields of BHK-21 cells cultured in Dulbecco’s Modified Eagle’s (DME)/high glucose medium supplemented with 10% serum. HyClone FetalClone II is a good alternative to FBS for BHK-21 cells.

Figure 4. Relative yields of CHO-K1 cells cultured in Ham’s F12 medium supplemented with 10% serum. HyClone FetalClone II, and HyClone FetalClone III, outperform FBS and are good alternatives to FBS for CHO-K1 cells.
Conclusions

The HyClone FetalClone products from Thermo Scientific are effective alternatives to FBS for promoting cell growth. They work well in their intended applications, and equally important, their supply is regular and predictable. The FetalClone products developed by Thermo Scientific are U.S.-sourced, low immunoglobulin products that find widespread use as an economical FBS substitute.

In addition to the HyClone FetalClone line of FBS alternatives, Thermo Scientific has also developed Thermo Scientific HyClone Bovine Growth Serum (BGS) as another FBS alternative. Like the HyClone FetalClone family, BGS is supplemented with a chemically defined, animal component free group of minerals and trace elements that enhances performance over FBS. Thermo Scientific is proud to be the provider of high quality FBS and FBS alternatives to meet the needs of the demanding cell culture market.

Figure 5. Relative yields of AIF cells cultured in Dulbecco’s Modified Eagle’s (DME)/high glucose medium supplemented with 10% serum. HyClone FetalClone I and Iron-Supplemented Calf Serum are good alternatives to FBS for AIF cells. Low immunoglobulins in HyClone FetalClone I are desirable for monoclonal antibody recovery.

Figure 6. Relative yields of NS0 cells cultured in RPMI-1640 medium supplemented with 10% serum. HyClone FetalClone I, HyClone FetalClone II, HyClone FetalClone III, and Iron-Supplemented Calf Serum, are all good alternatives to FBS for NS0 cells. The lower levels of immunoglobulins in the HyClone FetalClones are desirable for monoclonal antibody recovery.
Selected Publications citing Thermo Scientific HyClone FetalClone Products

FetalClone I

FetalClone II

FetalClone III