The Vero cell strain used in the following experiments comes from a flask of VERO IM 153 on the 153rd passage acquired from ATCC. This strain was divided into two sub-lines, one propagated in Fetal Bovine Serum (FBS)-supplemented medium and the other sub-line proliferated in FastGro™-supplemented serum-free medium.

Growth evaluation in FBS-supplemented versus FastGro™-supplemented medium

This study was performed with Vero cells adapted over long-term culture in serum-free, FastGro™-supplemented medium. Growth dynamics were compared to those of the sub-line cultured in FBS-supplemented medium. The results are from a 6-day culture period in T25 flasks. Cell numbers were determined at the end of the 144-hour culture period (Figure 1).

Medium	Seeding Cell Density (per cm^2)	Final Cell Density (per cm^2)	Cell Multiplication Index
Williams Medium E + 10% FBS	20,000	184,000	9.20
Williams Medium E + 10% FastGro™	20,000	197,000	9.85

Figure 1

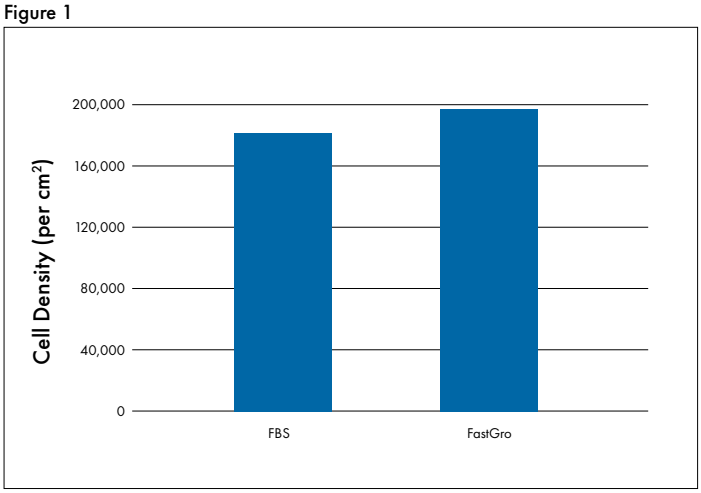
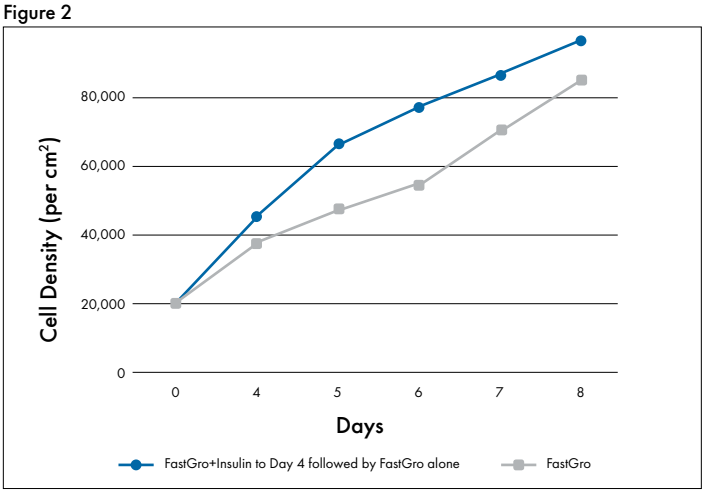


Figure 2



Growth of Vero cells in FastGro™- supplemented medium on microcarriers

In this study, a Vero strain was adapted over 3 passages to serum-free growth in FastGro™-supplemented Williams Medium E grown on microcarriers (200 mg cytodex/100 mL corresponding to 1200 cm^2 growth area) in small-scale laboratory bioreactors. To test for potential positive effects of insulin, the hormone was added to the culture medium (1.25 mg/L) of one group from day 0 through day 4, then switched back to Williams E + FastGro™ alone.

As can be observed, in both conditions the Vero cells showed a constant growth over the entire experimental period (Figure 2). The addition of insulin during the initial growth phase (4 days) caused a growth enhancement during the logarithmic growth phase, albeit the two experimental groups showed only a slight difference (< 10%) in the final cell numbers (< 10%). These results imply that a short mitogenic stimulus by insulin during the initial growth phase may be sufficient for generating a sustained growth benefit over the whole batch culture period.

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