Reducing Serum-Associated Contaminants from Growth Media in Primary Cell Culture

Application Note

Workflow Summary

Supplement basal media with ITSE + A to a final 1x-2x concentration. Reduce typical percentage of added FBS by 50-90%. Expand cells as normal in ITSE + A supplemented media for desired expansion time. Perform post-expansion cell count and doubling-time calculation.

Introduction

Fetal bovine serum contains an abundance of critical proteins and components that are crucial for promoting optimal cellular proliferation in ex vivo applications. For example, insulin promotes uptake of glucose and amino acids. Transferrin delivers intracellular iron, via the transferrin receptor, to drive cellular respiration. And albumin—the most abundant serum protein—delivers lipids for energy, has high antioxidant capacity, and binds to toxins, among other functions [1-4]. Other critical serum components include selenium, required for activity of various antioxidant enzymes, and ethanolamine, which is a phospholipid precursor.

However, increasing recognition of the risks of unwanted contaminants in serum sourced from animals has driven a push to reduce or eliminate the use of serum in primary cell-culture systems. To avoid loss of cell-culture performance associated with reducing or eliminating serum, it is necessary to add a defined supplement containing recombinant versions of the critical serum components discussed above. To address the functionality of growth media after serum reduction or replacement, InVitria has created a defined supplement containing the recombinant serum proteins insulin, transferrin, and albumin plus selenium and ethanolamine, incorporated in a convenient liquid concentrate called “ITSE + A”. This supplement contains all of the critical serum components in one solution. All of the protein components are synthetically manufactured using recombinant DNA technology in an animal-free host. This product enables the reduction of serum and its associated contaminants from primary cell culture and leads to improved ex vivo cellular analyses.

Materials

Growth Media Preparation

- ✔ Basal growth media of choice
- ✔ ITSE + A, 100x supplement (product no. 777ITS092)
- ✔ Fetal bovine serum
- ✔ Penicillin/streptomycin (optional)

Cells

- ✔ Tumor cell lines
- ✔ Primary cell lines

Cell Expansion & Subculturing

- ✔ Plates and/or flasks
- ✔ Dissociation solution (for adherent cell types only)
- ✔ Centrifuge
- ✔ Cell counter/hemocytometer

Protocol

Growth Media Preparation

1. Supplement ITSE + A into basal medium to a final 1x-2x concentration: For 1 liter of complete medium, add 10 mL of the concentrated 100x ITSE + A for 1x final concentration and 20 mL of concentrated 100x ITSE + A for 2x final concentration.
2. Supplement with fetal bovine serum (FBS) at a reduced percentage. Typical inclusion levels reported with ITSE + A are between 1% and 5% FBS final concentration, though this is dependent on the cell type and the lot of FBS being used.
3. If desired, add penicillin/streptomycin at 0.1x-0.5x final concentration.

Cells

1. Acquire desired cell type from ATCC or similar cell bank.
2. Thaw cells in recommended growth media containing 10% FBS and allow cells to grow until confluence has been achieved.

Cell Expansion and Subculturing

1. Harvest thawed cells from growth surface, if applicable, using 0.25% trypsin followed by a 1 in 10 washout dilution in growth media. If cells are suspension-type, collect the entire cell suspension.
2. Pellet cells by centrifugation and resuspend cells in the reduced-serum growth media supplemented with ITSE + A.
3. Subculture cells for standard subculture times following routine protocols and passage in the same reduced-serum growth media containing ITSE + A.
4. At each passage, harvest cells, take cell counts, and seed subsequent passages at desired cell density. Calculate doubling time as follows:

$$Doubling\ Time\ (hrs) = \frac{\ln(2)}{\ln\left(\frac{total\ viable\ cells}{initial\ total\ cells}\right) * \left(\frac{1}{days * 24}\right)}$$

Results and Discussion

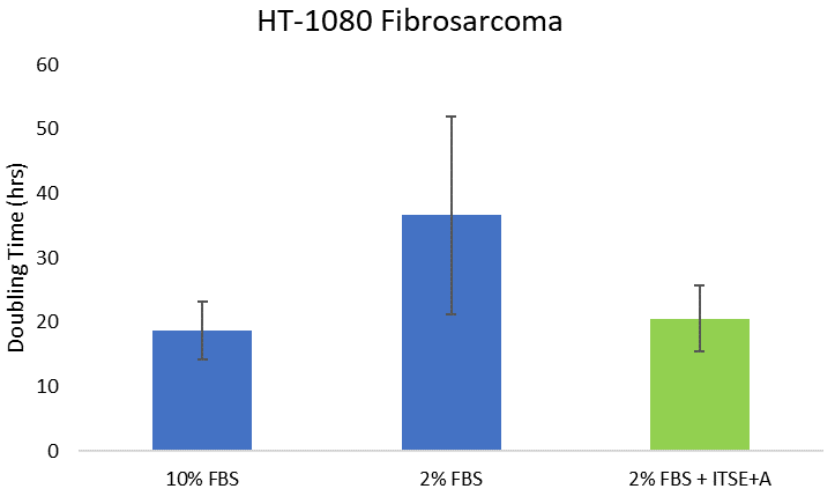

Figure 1. Supplementation of ITSE+A supported proliferation of HT-1080 cells following an 80% reduction in serum.

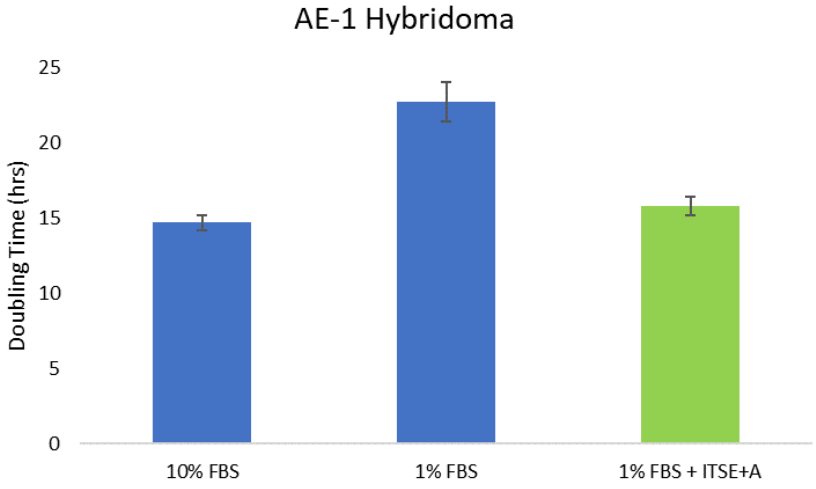

Figure 2. Supplementation of ITSE+A restored cellular proliferation of hybridoma cells following a 90% reduction in serum.

Reduced-serum conditions were tested for culture of fibrosarcoma and hybridoma cells in comparison to standard 10% FBS growth media. As shown by increased doubling time (i.e. slower growth), fibrosarcoma were sensitive to an 80% reduction in FBS; however, when ITSE + A was added to the reduced-serum media, their performance was comparable to cells cultured in 10% FBS (Figure 1). Similarly, hybridoma were able to proliferate even when serum was reduced by 90% when media was supplemented with ITSE + A (Figure 2). Combined, these results show that the addition of ITSE + A enables significant reduction in serum supplementation, for multiple cell types. By replacing the most well-known serum proteins and components with defined, recombinant sources, cell viability and proliferation can be restored in reduced-serum cell-culture applications.

References

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4. Saito, Y., Yoshida, Y., Akazawa, T., Takahashi, K., Niki, E. Cell death caused by selenium deficiency and protective effect of antioxidants. 2003. J. Biol. Chem. 278(41):39428-34.

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Atherly Pennybaker joined InVitria in 2017 as a Product Applications Specialist after receiving her Bachelor of Science in Chemical and Biological Engineering from Colorado State University. During her time there, she conducted undergraduate research in tissue engineering where she co-authored a paper on the osteoinductivity of an engineered biomimetic periosteum. She also worked on the design of a natural cell and tissue scaffold by means of electrospinning demineralized bone matrix. At InVitria she enjoys leveraging her scientific background to help customers successfully transition to blood-free, chemically defined systems.