



**ITSE+A™ Animal-Free** - Catalog Number: 777ITS092 Animal-Free, Chemically Defined, Sterile



# Introduction

The <u>ITSE+A<sup>™</sup> Animal-Free</u> (AF) synthetic supplement is a formulation designed to optimize cell culture conditions while reducing or eliminating the need for animal-derived components. This supplement combines five essential elements: Recombinant Insulin, Recombinant Transferrin, Selenium, Ethanolamine, and Recombinant Albumin.

Insulin, transferrin, selenium, and ethanolamine are widely recognized as critical factors for optimal cell growth in serum-free media<sup>1</sup>. These components work synergistically to provide cells with the necessary nutrients and growth factors typically found in serum-containing media. Ethanolamine is a phospholipid precursor that improves the performance of cells in serum free media<sup>2</sup> and is required for the growth of some cell types<sup>3</sup>. Insulin plays a multifaceted role in cell culture. Beyond its well-known function in glucose metabolism, insulin acts as a potent cell signaling molecule, promoting the uptake of glucose and amino acids. This hormone-like protein is essential for cell growth, protein synthesis, and lipid metabolism, making it indispensable in serum-free media formulations<sup>4</sup>. Transferrin is a non-toxic carrier of iron and reduces the generation of toxic free-radicals and peroxide<sup>5</sup>. Selenium is required for the activity of glutathione peroxidase, thioredoxin reductase, and other antioxidant enzymes<sup>6</sup>. Furthermore, our ITSE+A supplement intentionally contains albumin ("A") as an additional component. Albumin stabilizes pH, enhances nutrient availability and protects cells from stress-induced damage, making it a beneficial component in cell culture<sup>7</sup>.

By combining these essential components in a carefully optimized formulation, ITSE+A AF offers a comprehensive solution for researchers seeking to improve their cell culture outcomes while moving towards more defined, animal-component-free media systems.

# Thaw and Long Term Storage

It is recommended to store ITSE+A AF at -20°C, tightly sealed, and protected from light until use. When ready for use, thaw the product either at 37°C or at room temperature, while closely monitoring the process. Thawing time varies depending on the starting temperature and volume (10 mL, 100 mL, 1 L). Thawing times at room temperature can range approximately 1 hour (10 mL) to 10 hours (1 L), and thawing time for 37°C can range approximately 30 minutes (10 mL) to 6 hours (1 L). Time of thaw is variable to your process and therefore time deviations may occur. After thawing, a visible phase separation or turbidity may form at the bottom of the container. If this occurs, gently invert the container several times. Do not shake, swirl, or vortex the container to mix. Allow the product to sit at room temperature for 10-30 minutes, or until the solution becomes clear. Once clarity is achieved, store the product at 2-8°C for future use. ITSE+A is stable for 6 months after thaw when stored at 2-8°C.

# Instructions for Use

ITSE+A AF is prepared as a 100x sterile concentrate in Earle's balanced salt solution. The formulation is below:

Component	g/L (100x)	mg/L (In Use – 1x)
Recombinant human insulin	1.00	10.0
Recombinant human transferrin (Optiferrin <sup>®</sup> )	0.55	5.5
Sodium Selenite	6.70E-04	6.70E-02
Ethanolamine	0.20	0.002
Recombinant human albumin (Cellastim <sup>®</sup> -S)	20.00	200

ITSE+A AF may be used to reduce or eliminate serum, including Human AB Serum and Fetal Bovine Serum. For serum reduction, the serum reduction depends on the cell type. Some cell types may show additional benefit by supplementing media with ITSE+A AF at 2x final concentration. For best results, we recommend a dose titration with various inclusion levels to optimize according to your process flow and cell type.

ITSE+A AF supplement is often used to replace blood-derived (so called "Xeno-Free") versions of ITS+A and ITSE+A products. The components of InVitria's ITSE+A AF are defined and animal component free (no blood derived components). ITSE+A AF is made in the USA.

For further information or application of ITSE+A AF, please contact InVitria's technical support at 1-800-916-8311.

### References

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