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## ITS Animal-Free

### Guidelines for Use

#### ITS Animal Free™

777ITS091

Recombinant Insulin, Recombinant Transferrin, Selenium, 100x Supplement  
Blood-free

#### Introduction

Insulin, transferrin, and selenium are typically required for optimal cell growth in serum-free media<sup>1</sup>. Insulin has cell signaling functions and promotes the uptake of glucose and amino acids<sup>2</sup>. Transferrin is a non-toxic carrier of iron and reduces the generation of toxic free-radicals and peroxide<sup>3</sup>. Selenium is required for the activity of glutathione peroxidase, thioredoxin reductase, and other antioxidant enzymes<sup>4</sup>.

#### Long Term Storage

It is recommended to store ITS AF at 4°C, tightly sealed, and protected from light. ITS is stable for 1 year when stored as recommended.

#### Instructions for Use

ITS Animal-Free supplement is intended to replace blood-derived ITS and ITSE products. The components of ITS AF do not contain blood derived components. ITS AF is prepared as a 100x sterile concentrate in Earle's balanced salt solution. The formulation is below.

Component	g/L (100x)
Recombinant human insulin	1.00
Recombinat human transferrin (Optiferrin)	0.55
Sodium Selenite	6.70E-04

Use of ITS AF may be used to reduce or eliminate serum. For serum reduction, the degree depends on the cell type. For serum-free cell growth, InVitria recommends ITS AF in combination with Cellastim S. Some cell types may show additional benefit by supplementing media with ITS AF at 2x final concentration. For further information or application of ITS AF, please contact InVitria technical support at 1-800-916-8311.

#### References

1. Ozturk, S. S, Paulson, B.O. Effect of initial cell density on hybridoma growth, metabolism, and monoclonal antibody production. *J. Biotechnol.* 1990. 16:259-78.
2. Tsao, M.C., Walthall, B.J., Ham, R.G. 1982. Clonal growth of normal human epidermal keratinocytes in a defined medium. *J. Cell Physiol.*110(2):219-229.
3. Aisen, P. Iron in Biochemistry and Medicine, ed. Jacobs. A. and Worwood, M., Academic Press, New York., pp.87-129 (1980).
4. Saito Y, Yoshida Y, Akazawa T, Takahashi K, Niki E. 2003. Cell death caused by selenium deficiency and protective effect of antioxidants. *J. Biol. Chem.* 278(41):39428-34.

