

## Product Information

### Media Supplements:

### ITS, SITE, SPIT, SPITE, Fatty Acid-Albumin Supplements

#### INTRODUCTION

Most cells will not survive or exhibit optimal phenotypic properties for any length of time when cultured in basal medium alone. They require supplementation with additional growth and survival factors, such as hormones, transport proteins, trace elements or ECM factors. Traditionally, serum has been the supplement of choice to provide these factors. However, many investigators prefer to work in a serum-free culture environment to avoid the variability and contaminants that can be introduced by serum.

Serum-free formulations that substitute a purified form of the factors normally supplied by serum are suitable for many *in vitro* growth and differentiation studies. These factors include insulin, transferrin, selenium, pyruvate, and ethanolamine. Addition of other components varies greatly, depending on the cell type being studied and the basal medium employed.

#### ROLE OF COMPONENTS

**INSULIN** is a polypeptide hormone that promotes the uptake of glucose and amino acids and may owe observed mitogenic effect to this property.

**TRANSFERRIN** is an iron-transport protein. Iron is an essential trace element, but can be toxic in the free form. To nourish cells in culture, it is supplied bound to transferrin in serum.

**SELENIUM** is an essential trace element normally provided by serum.

**SODIUM PYRUVATE** has been shown to be beneficial as an additional energy source in some instances.

**ETHANOLAMINE** is a fatty acid that plays a significant role in the proliferation of hybridoma cells and frequently is added to supplements used for culturing these cells.

#### MEDIA SUPPLEMENTS

Nutritional studies indicate that the supplement components described are utilized by most mammalian cells. They enhance cell proliferation and decrease the serum requirement of many cell types. When the following supplements are used with 2 to 4 percent serum, proliferation is reported to be similar to medium supplemented with 10 percent serum.

**ITS** is a mixture of bovine insulin, human transferrin (partially iron-saturated), and sodium selenite. It is a general cell supplement designed for use in non-complex media (e.g. MEM, RPMI-1640) and complex media (e.g. Ham's F-12, DME/F-12, MEM) *with* sodium pyruvate.

**SITE** is a mixture of bovine insulin, human transferrin (partially iron-saturated), sodium selenite, and ethanolamine. It is a general cell supplement designed for use in non-complex media (e.g. MEM, RPMI-1640) and complex media (e.g. Ham's F-12, DME/F-12, MEM) *with* sodium pyruvate.

**SPIT** is a mixture of bovine insulin, human transferrin (partially iron-saturated), sodium selenite, and sodium pyruvate. It is designed for cell cultures in which media *without* sodium pyruvate are used.

**SPITE** is a mixture of bovine insulin, human transferrin (partially iron-saturated), sodium selenite, sodium pyruvate and ethanolamine. It is designed for cell cultures in which media *without* sodium pyruvate are used.

**FATTY ACID-ALBUMIN** complexes have been employed as alternative sources of lipids in the development of serum-free media. Fatty acids bind to serum proteins in high proportions. Such proteins may release beneficial fatty acids and bind those that are inhibitory. Oleic acid bound to BSA has been shown to be beneficial to the growth of a variety of cell types (e.g. BHK, hybridoma). Similar observations have been made regarding linoleic acid, a precursor of prostaglandins. A mixture of poly-unsaturated and monosaturated fatty acids (i.e. linoleic acid and oleic acid) used as a medium supplement has been reported to exhibit a synergistic effect.

### MEDIUM SUPPLEMENT FORMULATION TABLE

Final Conc. [1X]	ITS I 1884	SPIT S 5791	SPITE S 5666	SITE S 4920	ITS I 3146	ITS +1 I 2521	ITS +2 I 2646	ITS +3 I 2771	SITE +1 S 5045	SITE +2 S 5170	SITE +3 S 5295	FAC +LO L 9655	FAC +L L 9530	FAC +O O 3008
insulin mg/L	5	10	10	10	10	10	10	10	10	10	10	—	—	—
transferrin mg/L	5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	—	—	—
selenium µg/L	5	5	5	5	5	5	5	5	5	5	5	—	—	—
pyruvate mg/L	—	110	110	—	—	—	—	—	—	—	—	—	—	—
ethanolamine mg/L	—	—	2	2	—	—	—	—	2	2	2	—	—	—
BSA mg/L	—	—	—	—	—	0.5	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0
linoleic acid µg/mL	—	—	—	—	—	4.7	—	4.7	4.7	—	4.7	9.4	9.4	—
oleic acid µg/mL	—	—	—	—	—	—	4.7	4.7	—	4.7	4.7	9.4	—	9.4

NOTE: These products are sold at [100X] concentration. The concentration in the vial is [100X] value shown in the Table.

### REFERENCES

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