Product Profile

Product Name:	*Grace's Insect Cell Medium with L-Glutamine*
Product Catalog Number	*01-155-1*
Unit Size Availability:	(A)500ml;(B)100ml
Formulation:	Clear, Colorless Solution
Specified Storage Conditions:	2-8°C
Stability: (Under Specified Handling & Storage)	*Please Refer to the Product Label*

Important Note! Please read the ***MSDS*** and ***Product Profile*** carefully in their entirety ***before*** using this material for possible safety precautions and potential hazards.

Product Description:

Grace's Insect Cell Medium with L-Glutamine when properly supplemented can be utilized for growth and maintenance of insect cells in culture such as in Dipteran and/or Lepidopteran cell lines. It has also been successfully used on a variety of other insect cell types as well as for the production of recombinant proteins *via* the Baculovirus Protein Expression System (BPES). Many types of insect culture media have been formulated to imitate or mimic the diverse biochemical properties characteristic of insect hemolymph for the study of different biological processes. It should be obvious that variegated and diverse formulas have been developed or rather designed to endeavor to meet individual, unique niche requirements, but nevertheless, most often differ both quantitatively and qualitatively in terms of constituents.

The application of insect cell culture for heterologous protein expression has progressively increased over the last several decades. An important factor underscoring this popularity of insect cell expression is the innate ability of insect cells to produce relatively large quantities of post-translationally modified eukaryotic proteins in a relatively short period of time.

Grace's medium was originally developed almost half a century ago to support the growth of the Australian Emperor Gum Moth (*Opodiphthera eucalypti*) cells. It is a modification of Wyatt's medium which was formulated to resemble the biochemical profile of hemolymph from *Bombyx mori* (the Domesticated Silkworm Moth). Grace was the first to establish continuous cell lines using this medium. Prior to use, Grace's medium is typically supplemented in varying amounts and combinations based on individual niche requirements.

Grace's Insect Cell Medium with L-Glutamine is a medium designed and may be optimized for the culture of *Lepidopteran spp.* insect cells with the addition of serum. (Class, *Insecta*; Order, *Lepidoptera*; Family *Noctuidae*). The medium supports the growth and maintenance of both anchorage-dependent and suspension cultures of Sf-9 cells derived from the pupal ovarian tissue of the Fall Armyworm, *Spodoptera frugiperda* (J.E.Smith). The Sf-9 cell line is commonly used to isolate and propagate recombinant Baculoviral stocks and for the production of recombinant proteins. *Grace's Insect Cell Medium with L-Glutamine* is primarily used as a basal medium for the growth and maintenance of cell lines derived from Lepidopteran species and that when supplemented with either Fetal Bovine Serum (FBS), and/or a combination of Lactalbumin Hydrolysate, Yeastolate or Yeast Extract, Bovine Serum Albumin(BSA) or other protein sources, provide excellent results.

For example, the Sf-9 cell line is highly susceptible to infection with the Baculovirus, *Autographa californica* multicapsid nuclear polyhedrosis virus (AcMNPV). The Baculoviridae are a unique group of eukaryotic viruses that parasitize insects with the prototypic member of that family being, AcMNPV and therefore, can be utilized with all Baculovirus Expression Vectors. The diversity of AcMNPV-based transfer vectors establishes Baculovirus expression as a preferred system for high level functional eukaryotic gene expression and the large-scale production of recombinant proteins.

The Nucleopolyhedrovirus (NPV) which is one genus of Baculoviridae, contains single (SNPV) or multiple (MNPV) nucleocapsids per envelope. The enveloped virions are protected in a polygonal-structured capsid, by which it enables the virus to penetrate and infect not only susceptible cells but also to enhance the viral reproduction process. Once the host is compromised, the viral occlusions that contain virions are released. Since this occlusion-derived virus form (ODV) of the disease is responsible for the primary infection by horizontal transmission of AcMNPV in nature, it must be emphasized that the following effects such as: polyhedrin biosynthesis, nuclear localization and normal polyhedron assembly are all important events within the viral replication cycle and are thus part and parcel of the normal assembly of viral occlusions.

The virus, isolated from the Alfalfa looper, (*Autographa californica*-Speyer), is utilized for the Recombinant Expression Vector System (BEVS). Insect cells, infected with the virus, display accumulations of the highly expressed, major viral-encoded structural protein, polyhedrin. This protein forms the crystalline matrix of viral polyhedral bodies also known as polyhedra which are crucial for viral infection of insects in the wild. As aforementioned, they are a major component of the nuclear viral occlusions produced during replication of AcMNPV within multiple embedded virions. Although polyhedrin is an extremely important protein for viral propagation and survival in the wild, under laboratory conditions, however, where growth parameters are tailored to suit the virus and not the other way around, the polyhedron matrix is no longer a pre-condition for virus survival. Thusly, the *polh* gene can be replaced with a heterologous gene of choice so forth and so on. These hypertranscribed promoters are thus part and parcel of the BEVS.

Baculovirus Expression Vector System (BEVS)
The Baculovirus Expression Vector System (BEVS) is based upon the fact that non-essential polyhedron protein is produced in large quantities. We see that the Molecular Biologist has exploited this discovery by substituting the polyhedrin gene with a gene of choice utilizing conventional recombination techniques. This introduction of a foreign gene into a non-essential region of the viral genome by way of homologous recombination with a transfer vector containing the cloned gene is an event that occurs in co-transfected insect cells. The production of foreign protein is then achieved by infection of additional insect cell cultures with the resultant recombinant virus. *In vitro* replication and infection does not require the production of polyhedron protein as cell-to-cell infection occurs via the (extracellular/budded virus particles) or ECV's which led to the development of BEVS. In cell culture, the production of BEVS was designed for heterologous gene expression that provides correct folding of:

- Disulfide Bond Formation
- Recombinant Protein
- Oligomerization
- Other Post-Translational Modifications(e.g. Signal, Proteolytic Ceavage, N & O-glycosalation)

As a result, a major advantage is the quick turnaround time for recombinant protein expression as the overexpressed protein exhibits the anticipated biological activity, immunogenicity and antigenicity similar to the authentic natural proteins.

Serum and Serum Products
Serum or serum-like replacements are necessary for the growth and proliferation of cells. . Serum is largely undefined, but it supplies a mixture of all types of proteins, structural, carrier and functional proteins including essential growth factors, hormones, minerals, trace elements and even inhibitory substances. When supplemented with Fetal Bovine Serum (FBS), *Grace's Insect Cell Medium with L-Glutamine* has been found to support the growth and proliferation of both primary and established insect cells in culture. Serum supplementation is a crucial planning step which plays a vital role in the success of your final medium. ***Biological Industries'*** Pre-Screened and Pre-tested Serum undergoes the most stringent and rigorous Quality Control/Assurance standards and protocols testing all raw materials and finished products in order to meet the demands of international markets and ensure high quality and consistency. All our serum products meet approved compliance validation and specifications prior to use and or release of the final product to the end-user. Our Fetal Bovine Serum (FBS) undergoes a methodical and comprehensive battery of Physico-Chemical, Microbiological and Biological Performance Testing Procedures. Each batch is traceable, well-documented from source of origin through the thorough and systematic Quality Control process. All documentation and certification are available upon request.

These more complex media not only meet the minimum requirements for cell growth and proliferation but also are part and parcel of a much wider array of factors culminating in a final medium that segues with the essential cell-niche requirements demanded for optimal results.

For example the addition of L-Glutamine, a precursor of glutamate, is one of the most readily available sources of energy for many rapidly dividing cell-types for use *in vitro* and is a key component and essential amino acid that is required in many cell-culture media formulations and in virtually all mammalian cells in culture. Sodium pyruvate may also serve as an additional an easily accessible carbohydrate energy source for cells in culture. These balanced energy sources serve as carbon skeletons for cell anabolic processes in addition to nucleic acid metabolism and protein production while limiting the potential cumulative build-up effects of toxic levels of ammonia.

The selection of a specific and complex nutrient-enriched medium such as *Grace's Insect Cell Medium with L-Glutamine* typically represents the requirements for promoting cell growth and maintenance, is multi-faceted and is based as well upon several major fundamental characteristics:

- Cell Type
- Type of Cell Culture Environment (e.g., Clonal, Monolayer or Suspension)
- Uniquely Defined Individual Niche Requirements

Grace's Insect Cell Medium with L-Glutamine also contains constituents that include a typical and wide variety of, among others:

- Amino Acids
- Glucose
- Sucrose
- Sodium Bicarbonate
- Inorganic Salts
- Vitamins
- Trace Elements

Some Predominant Characteristics of *Grace's Insect Cell Medium with L-Glutamine* include:

- Ready-To-Use-Formulation after Appropriate Supplementation
- With L-Glutamine
- Without Insect Hemolymph
- Without Lactalbumin Hydrolysate
- Without Yeastolate
- More Precise Evaluation of Cell Function
- Improves Cell Adaptation Time
- Promotes Cell Performance and Productivity
- More Uniform & Consistent Media Performance
- Sterile-Filtered(0.1µ)

Storage & Handling Precautions and Disclaimer:
For *in vitro* diagnostic use only.

Grace's Insect Cell Medium with L-Glutamine should be stored under defined conditions between 2-8°C. The product should not be left in the light for prolonged periods as it is light-sensitive. When stored in the dark under ideal conditions, the product is stable until the expiry date on the label.

As with any other liquid media formulations,***deterioration of liquid media*** may be recognized by any of the following characteristics, among others including: (a). color change, (b). presence of clumping/flocculent debris/ granulation/ particulates\ precipitates or sediments (c). insolubility,(d). and/or decrease in expected performance parameters. Any material described above should not be used and therefore discarded.

Instructions/Procedure:

1) Take a bottle from the proper storage conditions between 2-8°C and read the label.
2) Allow to warm to room temperature prior to use.
3) Ensure that the cap of the bottle is tight.
4) Gently swirl the solution in the bottle to ensure homogeneity.
5) Wipe the outside of the bottle with a disinfectant solution such as 70% ethanol.
6) Using aseptic/sterile technique under a laminar-flow culture hood, work according to established protocols.

Quality Control

Test	Specification
Appearance:	Clear, Colorless Solution
Osmolality:	300-340 mOsm/kg
pH:	6.0-6.6
Sterility:	Sterile

Auxiliary Products

Product Name	Catalog Number	Storage Temperature
Lactalbumin Hydrolysate Solution,50X Concentrate	01-356-1B	2-8°C
Yeastolate Solution,50X Concentrate	01-357-1B	2-8°C
Alanyl-Glutamine Solution(A Dipeptide Substitute)	03-022-1	-20°C
Penicillin-Streptomycin Solution	03-031-1	-20°C
Sodium Pyruvate Solution	03-042-1	-20°C
BioInsect-1 with L-Glutamine	05-050-1	2-8°C
Note: For a list of Serum, or other Antibiotics, please refer to our Product Catalog/Product Profiles/Guides and Internet Site.		

References:

1) Biological Industries (BI)Specifications
2) Darling, D.C. and Morgan S.J. Animal Cells: Culture and Media, John Wiley & Sons, New York, 1994
3) Lackie, J. M. The Dictionary of Cell & Molecular Biology, Academic Press: London, 2007