

Product Profile

Product Name:	Nutrient Mixture F-12(Ham), with L-Glutamine
Product Catalog Number	01-095-1
Unit Size Availability:	(A)500ml ;(B)100ml
Concentration:	1X
Formulation:	Clear 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:

The series of Ham's Nutrient Mixtures of which Nutrient Mixture F-10,F-12 and other modifications are a part with L-Glutamine supplementation, was originally designed and developed specifically for supporting the growth of several clones of Chinese Hamster Ovary (CHO), HeLa clones and Mouse L-Cells. Both Nutrient Mixtures were developed with the intention that serum supplementation may be used as an option in serum-containing media or in a serum-free growth environment depending upon the individual cell culture. Whereas and on the one hand ,Ham's F-10 is known not only to support primary explants of chicken, rabbit and rat tissues, but also the growth of White Blood Cells(WBC's) for chromosomal analysis and the growth of human diploid cells. And on the other, *Nutrient Mixture F-12(Ham's), with L-Glutamine* was designed and developed specifically for the growth of Primary Rat Hepatocytes (PRHs) and Rat Prostate Epithelial Cells (RPECs).F-12, as the medium of choice for a Clonal Toxicity Assay (CTA) has been utilized with Chinese Hamster Ovary (CHO) cells.

CHO cells are the most widely utilized Mammalian hosts for the industrial production of recombinant protein therapeutics which includes transfection, expression and large-scale recombinant protein production. They are not only used in gene expression and nutrition, but also in genetics, toxicity screening and in particular when expressing recombinant proteins. In short, it is the most commonly used cell production line in Genetic Engineering today. HeLa cells are Transformed Human Epithelial Cells (THECs), highly stable, so-called "immortal" cell line isolated from a cervical carcinoma propagated and later commercialized for scientific research. Termed "immortal" in that they have the unusual property of being able to divide indefinitely in cell culture (i.e. when the characteristic cell milieu is optimally sustained) unlike primary and secondary cells, these unique "cancerous" cells possess features unlike other tumor cells in that they possess 82 chromosomes and possess several copies of the same chromosome induced by chromosomal mutations. Transformed cells do not necessarily mean that these are cancer or tumor cells, although they may become cancerous or tumorigenic. Most simply, they are cells whose growth properties that have been altered in some way.

Most common types of media consist of an isotonic, buffered basal nutrient enriched environment which provides an energy source, inorganic salts, vitamins, amino acids as well as additional constituents (e.g. supplements) according to the demands of a particular cell line. This relatively more complex medium formulation provides the optimal cell-culture environment in which *in vitro* culture conditions mimic those of the *in vivo* environment including basic nutritional requirements, osmotic pressure, physiological pH, temperature among other considerations. At a minimum, it consists of the foundation medium components that are all part and parcel of a pre-tested complete media to assist the cells in integrating their metabolic demands.

Nutrient Mixture F-12(Ham's), with L-Glutamine contains no growth-promoting factors or antimicrobials. The type of medium recommended usually is dependent upon the type and character of the cells in culture. Supplementation is the process in which specific additions or supplements (e.g. growth factors, serum, fatty-acids, buffers, hormones) compliment a typical basal or balanced salt solution medium or more complex media such as RPMI 1640.

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.

Function of L-Glutamine

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. The addition of Sodium pyruvate serves as an additional an easily accessible carbohydrate energy source for cells in culture. Along with D-glucose, 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 Function of Sodium Bicarbonate

To maximize success in cell culture, the *in vitro* culture conditions are created to mimic the *in vivo* conditions of osmolality, pH, temperature and nutrition. Ions such as HCO_3^- and Na^+ , among others are the major contributors to the osmolality of cell culture media. HCO_3^- levels are determined by the concentration of CO_2 in the incubator (i.e. in contact with the growth medium). Sodium Bicarbonate (NaHCO_3) and CO_2 buffering is probably the most popular system used which requires a CO_2 level of 5-10 % (i.e. dependent on the media utilized) and 100% humidity.

Culture media are often buffered to compensate for the cellular production of CO_2 and Lactic Acid as by-products of metabolism. Traditionally, Basal Cell Culture media have been buffered by HCO_3^- (Bicarbonate). As cells grow, CO_2 evolves; the dissolved CO_2 forms a buffering system with the bicarbonate. However, if cell density is low or the cells have entered into the so-called "Lag Phase," they may not produce sufficient CO_2 to maintain optimal pH and to counter these potential problems, Bicarbonate-Buffered media require the use of incubators with a 5-10% CO_2 atmosphere. Media with low levels of bicarbonate (HCO_3^-) on the one hand, such as MEM(@1.5-2.2g/l) require ~5% CO_2 ; DMEM with higher levels of bicarbonate (i.e., 3.7g/L) on the other, require 10% CO_2 in order to maintain the correct pH level. The most important factor in utilizing the correct percent CO_2 is based upon the medium's bicarbonate level to maintain physiological pH, which is irrespective of cell type.

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. 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.

Nutrient Mixture F-12(Ham's), with L-Glutamine contains numerous important basic constituents in a ready-to-use formulation, which includes a typical and wide variety of elements, among others:

- ◆ Amino Acids
- ◆ Vitamins
- ◆ Inorganic Salts
- ◆ Phenol Red
- ◆ Trace Elements

Some Predominant Characteristics of *Nutrient Mixture F-12(Ham's), with L-Glutamine* includes:

- § Liquid Formulation
- § With D-Glucose
- § With Phenol Red($\text{C}_{19}\text{H}_{13}\text{NaO}_5\text{S}$) as a pH indicator
- § With L-Glutamine
- § With Sodium Pyruvate
- § With Sodium Bicarbonate(NaHCO_3)
- § Promotes Cell Performance and Productivity
- § More Uniform & Consistent Media Performance
- § Sterile-Filtered(0.1μ), Cell-Culture-Tested

Storage, Handling, Stability Precautions and Disclaimer:

For *in vitro* diagnostic use only.

Nutrient Mixture F-12(Ham's), with L-Glutamine is stable when stored under defined conditions at 2-8°C. The product is light-sensitive and therefore should not be left in the light. When stored in the dark under ideal conditions, the product is stable until the expiry date.

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 defined storage conditions at 2-8°C and read the label. Warm to Room Temperature (15-30° C) prior to use.
- 2) Ensure that the bottle cap is tight and swirl the bottle for complete homogeneity.
- 3) Wipe the outside of the bottle with a disinfectant solution such as 70% ethanol.
- 4) Using aseptic/sterile technique under a laminar-flow culture hood, work according to established protocols.

Quality Control:

Test	Specification
Appearance:	Clear Solution
Cell Culture:	Pass Test
Cell Line:	A-549
Endotoxins:	Test and Record
Osmolality:	282-312 mOsm/kg
pH :	7.0-7.5
Sterility:	Sterile

Auxiliary Products

Product Name	Catalog Number	Storage Temperature
Nutrient Mixture F-10(Ham's) ,with L-Glutamine	01-090-1	2-8°C
Nutrient Mixture F-12(Ham's),10X Conc., with L-Glutamine, without Sodium Bicarbonate	01-090-5	2-8°C
Nutrient Mixture F-12(Ham's), with L-Glutamine, without Sodium Bicarbonate	01-095-5	2-8°C
RPMI 1640, with L-Glutamine	01-100-1	2-8°C
RPMI 1640, without D-Glucose, without L-Glutamine	01-101-1	2-8°C
RPMI 1640, without Phenol Red, without L-Glutamine	01-103-1	2-8°C
RPMI 1640, without L-Glutamine	01-104-1	2-8°C
RPMI 1640 10XConc., without L-Glutamine, without Sodium Bicarbonate	01-104-5	2-8°C
Dulbecco's Phosphate Buffered Saline(DPBS) without Calcium and Magnesium	02-023-1	15-30°C
L-Glutamine Solution 29.2mg/ml in Saline	03-020-1	-20°C
L-Alanyl-L-Glutamine Solution(A Dipeptide Substitute)	03-022-1	-20°C
Penicillin-Streptomycin Solution	03-031-1	-20°C
Sodium Pyruvate	03-042-1	-20°C
Water, Cell Culture Grade	03-055-1	15-30°C
Fetal Bovine Serum	04-001-1	-20°C
Fetal Bovine Serum(Qualified for Human Embryonic Stem Cells)	04-002-1	-20°C
Adult Bovine Serum	04-003-1	-20°C
Donor Horse Serum	04-004-1	-20°C
Fetal Bovine Serum(TET System Approved)	04-005-1	-20°C
Porcine Serum	04-006-1	-20°C
European Grade Fetal Bovine Serum	04-007-1	-20°C
Rabbit Serum	04-008-1	-20°C
Donor Goat Serum	04-009-1	-20°C
Fetal Bovine Serum(Dialyzed)	04-011-1	-20°C
Special Newborn Calf Serum(Less than 10 days of age)	04-102-1	-20°C
Fetal Bovine Serum(Heat-Inactivated)	04-121-1	-20°C
Special Newborn Calf Serum(Heat-Inactivated)	04-122-1	-20°C
Adult Bovine Serum(Heat Inactivated)	04-123-1	-20°C
Note: For a list of other Antibiotics, Serum, or other Reagents & Supplements, please refer to our Product Catalog/Product Profiles/ Product 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