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Product Profile

Product Name:	Minimum Essential Medium Eagle (MEM-NEAA,	
	Earle's Salts Base, with Non-Essential Amino Acids),	
	without L-Glutamine	
Product Catalog Number	01-040-1	
Concentration:	1X	
Unit Size Availability:	(A)500ml (B)100ml	
Formulation:	Clear Red-Colored Solution	
Optimal Storage Conditions:	2-8°C	
Stability: (Under Specified Handling & Storage	Please Refer To Product Label	
Conditions)		

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:

Minimum Essential Medium-Eagle (MEM-NEAA), Earle's Salts Base, with Non-Essential Amino Acids, without L-Glutamine is one of the most widely utilized of all synthetic growth media. MEM has been used for the cultivation of a wide variety of cells grown in monolayers. Optional supplementation of Non-Essential Amino Acids (NEAA's) to the formulations that incorporate either Hank's or Eagle's salts has broadened the utility of this medium.

Living organisms differ considerably with respect to their ability to synthesize Amino Acids and the forms of nitrogen which they may utilize for such a purpose. The higher vertebrates do not possess the ability to synthesize all the common Amino Acids like some of the more versatile higher plants or microorganisms like *E. coli* that can. Humans and the albino rat can make only ten(10) of the twenty(20) AA's required for protein synthesis. These collectively are known as the Non-Essential Amino Acids (NEAA's), the remainder being the Essential Amino Acids (EAA's). Amino acids are the building blocks (i.e. linear chains of amino acids) of proteins and proteins have a variety of functions in metabolism, may be precursors for the biosynthesis of other biological molecules, function with coenzymes, are critical to life and are therefore needed by every living organism. An amino acid is a molecule containing both amine and carboxyl functional groups. Proteins are chains of amino acids linked together by peptide bonds. Chemically speaking, they are carboxylic acids which have an amine group attached to it. Next to water, protein makes up the largest portion of our body weight as it is contained in all the muscles, body organs, hair, nails and other body systems. There are more than 500 amino acids which occur in nature of which humans can produce 10 of 20 amino acids; the others must be supplied by the diet. Unlike fat and complex carbohydrates, the human body does not store excess amino acids as a reserve; they must be supplied in the diet every day. Concentrated supplements like MEM NEAA's 100X add nutrients that cells might not encounter thereby reducing the overall biosynthetic burden *to* cell cultures *in vitro*.

Amino acids are incorporated into proteins. At a minimum, basal medium must contain those essential amino acids (EAA's) that cannot be synthesized by the cells including L-Cysteine and L-Tyrosine at a rate to meet the metabolic requirements of the cells in culture. Individual requirements vary for the cell type being cultured. Some more specialized media often have non-essential amino acids (NEAA's) added to ensure that amino acids do not limit the maximum cell concentration attainable in order to compensate a particular cell type which is unable to manufacture them of if they leach rapidly into the medium.

As the selection of a nutrient medium or supplementation thereof is strongly influenced, among others, by many factors, of note are three major considerations:

- ♦ Cell Type
- Type of Culture(e.g., Clonal, Monolayer, Suspension)
- Degree of Chemical Definition

It is recommended to review the extensive literature concerning cell-culture media and its supplementation and the physiological parameters required for each specific cell-line as per their essential niche requirements.

All media consist of an isotonic, buffered, basal nutrient medium providing an energy source along with amino acids, inorganic salts, vitamins and other supplements. All these basic components in addition to other various supplements are part and parcel of a final formulation that segues into a unique and integrated composition that differs only in terms of concentration and proportion of the basic constituents which essentially characterizes each media type. The choice of media usually depends upon the type of cells in culture and MEM is one of the more common and simpler formulations like RPMI or DMEM in contrast to the more complex and/or enriched media such as Iscove's, Ham's F-12 or CMRL to name a few. MEM was originally developed by Harry Eagle as a result of his research endeavors to determine the essential nutrient requirements of mouse L-cells and HeLa cells in culture. MEM is one of the most commonly used of all synthetic cell culture media, and refers to only one of several formulae developed by Eagle to support transformed HeLa cells in monolayer culture. HeLa denotes those epithelial tumor cells originally derived from the first continuously human cervical-cultured carcinoma strain propagated and later commercialized.

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MEM is simply a modification of BME containing higher concentrations of essential nutrients. Subsequent studies by Eagle and other researchers determined that if additions were made to the original BME, it could aid and support a much wider spectrum of more fastidious cells in culture. MEM incorporates these modifications and thus includes, among others constituents, higher concentrations of Amino Acids so as to more closely approximate the protein composition of Mammalian cells in culture. MEM has been utilized for the cultivation of a wide array of cells grown in monolayers. The optional supplementation of non-essential Amino Acids (NEAA's) to the formulations that incorporate either Hank's or Eagle's Salts has broadened the usefulness of this medium.

At the minimum, MEM-NEAA (Earle's) Culture Media consists of NEAA'a amino acids, energy sources, inorganic salts, and vitamins among other nutrients. It is basically an unsupplemented medium which promotes the growth of many types of cells which do not require any special nutrients. Development of a Basal Culture Medium is a prerequisite for the attachment, spreading and growth of cells *in vitro*. To maximize success, the *in vitro* culture conditions are designed to mimic such crucial *in vivo* conditions of nutrition, osmolality, pH and temperature. Optimal and critical nutrient components including such inorganic salts (e.g.NaCl, KCl, CaCl₂), amino acids (e.g. Arginine, Histidine, Lysine), energy sources (e.g. Glucose), and vitamins (e.g. Folic Acid, Thiamine, Riboflavin) are part and parcel that culminate in a perfect milieu for growth and viability.

MEM-NEAA (Earle's) is one of a variegated and similar array of cell culture media that form the basis of many complex media formulations. It is a non-complex medium well-suited for a wide range of Mammalian cells when utilized with serum supplementation. These variegated inorganic salt solutions have been developed in order to fulfill the basic cell requirements for five basic and essential ions including: calcium, magnesium, phosphate, potassium and sodium and therefore contain various amounts of CaC_k, KCI, MgSO₄, NaCI, NaHCO₃, NaH₂PO₄ and other salts, among others. The key constituents of salts are the ions which function in osmolality whereas others such as Calcium and Magnesium are known, among other functions, to serve as cofactors for and support cell attachment and aggregation. D-Glucose serves as a major carbon and energy source. Sodium Bicarbonate has an intimate relationship with and plays a major role with CO₂ by helping to maintain optimal physiological pH.

MEM Eagle was developed as a modification of the original BME (Eagle's Basal Medium) with Earle's Salts. Earle's like Hank's has been part and parcel of cell culture since the early 1950's and has since been modified and enriched with a diverse complex of other salt compounds, along with D-Glucose with or without Phenol Red that eventually segue into a final medium based upon application and technique to meet the final cell culture medium's unique niche requirements. Earle's Salts comprise a higher concentration of bicarbonate and different concentrations of salts of the essential inorganic ions (e.g., NaCl, CaCl₂, KH₂PO₄).

Biological Industries' extensive array of similar salt solutions is widely used as an inorganic base in the preparation of media for cell culture and available according to application and technique. In order to optimize success, the eventual *in vitro* cell culture conditions must ultimately mimic the *in vivo* conditions for adequate cell attachment, membrane potential, coenzyme factors, osmotic pressure, and physiological pH. The final cell culture medium determined by the researcher must provide the proper milieu whose primary responsibility lies with these salt solutions which vary in terms of concentration and complexity.

Cultured cells require a sterile environment and an optimal nutrient supply for growth and viability. Over the years variously defined basal media have been designed, developed, modified and enriched with a wide spectrum of constituents for supporting a vast range of cell types. Precise media formulations have been specifically developed by optimizing the concentrations of each and every component which performs a uniquely defined function.

Unlike the balanced salt solutions that form the basis of many complex media formulations and are utilized to maintain cells for the short-term in a viable condition, MEM-NEAA may be modified and further enriched to promote the growth and viability of cells in culture. MEM-NEAA may be utilized for a broad and variegated spectrum of cell lines when properly supplemented. For some applications, Serum supplementation is required to supply essential growth factors, hormones providing additional sources of Carbohydrates, Amino Acids and Vitamins.

Monolayer Culture

Cell Monolayering (CM) is that tendency for animal tissue cells growing on solid surfaces (i.e. *in vitro*) to cover the complete surface area with a complete layer one-cell thick before growing on top of one another. This non-random distribution is generated by contact inhibition of locomotion, a phenomenon in which colliding cells change direction rather than move over one another. Of the theories why some but by no means all types of cells stop growing when a monolayer is formed, demonstrate that present evidence seems to favor limitation of the supply of growth factors from the medium, rather than by any inhibitory effect of contact on growth. Monolayer cultures are essential for anchorage-dependent cells (ADC's). ADC's require biologically inert, non-toxic and optically transparent surfaces that allow cells to attach and allow movement for growth. The so-called "scaling-up" of such cultures is based on increasing surface area by utilizing plates, spirals, ceramics and micro-carriers.



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Some Predominant Characteristics of ME M-NEAA (Earle's) without L-Glutamine include:

- Liquid Formulation
- With Non-Essential Amino Acids
- With Earle's Salts
- With Sodium Bicarbonate(NaHCO₃)
- With Phenol Red(C19H13NaO5S) as pH indicator
- Sterile-Filtered(0.1µ)
- Cell Culture-Tested

Storage & Stability:

The product should be stored at 2-8°C. The medium should be warmed to room temperature prior to use. 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. Instructions/ Procedure:

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

Quality Control:

Test	Specification
Cell Culture Test::	Pass Test
Cell Line:	BSC-1
Endotoxins:	Test and Record
Osmolality:	280-302 mOsm/Kg
pH:	7.2-7.5
Sterility:	Sterile

Auxiliary Products:

Product Name	Catalog Number	Storage Temperature
Basal Medium Eagle (BME), Earle's Salts Base, without L-Glutamine,	01-015-5	2-8°C
without Sodium Bicarbonate 10X		
Minimum Essential Medium Eagle (MEM-E), Earle's Salts Base,	01-025-1	2-8°C
without L-Glutamine		
Minimum Essential Medium Eagle (MEM-H), Hank's Salts Base,	01-035-1	2-8°C
without L-Glutamine		
Minimum Essential Medium -Alpha (MEM-A), with 1g/I D-	01-042-1	2-8°C
Glucose(Low Glucose), with L-Glutamine, without Ribonucleosides		
and Deoxyribonucleosides		
Minimum Essential Medium -Alpha (MEM-A), with 4.5g/I D-Glucose	01-043-1	2-8°C
(High Glucose), with L-Glutamine, without Ribonucleosides and		
Deoxyribonucleosides		
Minimum Essential Medium(MEM) for Suspension Cultures, without	01-045-1	2-8°C
	04,000,4	0.000
Medium-M-199(Earle's), Earle's Salts Base, with L-Glutamine	01-080-1	2-8°C
Medium-M-199 10X Conc., (Earle's), Earle's Salts Base, with L-	01-080-5	2-8°C
Giutamine, without Sodium Bicarbonate	02.010.5	D
Earle's Balance Salt Solution TUX Conc., without Sodium Bicarbonate	02-010-5	Room Temperature(15-30°)
Earle's Balance Salt Solution without Phenol Red	02-011-1	Room Temperature(15-30°)
Earle's Balance Salt Solution without Phenol Red, without Sodium	02-011-5	Room Temperature
Bicarbonate		(15-30°)
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,10,000 units/ml Penicillin G Sodium	03-031-1	-20°C
Salt,10mg/ml Streptomycin		
Sterile Culture-Grade Water	03-055-1	Room Temperature(15-30°)
Serum-Free Cell Freezing Medium	05-065-1	2-8°C
Note: For a list of other Antibiotics, Serum, Reagents and	used and the second statements	and the state of the second state of the sta
Supplements, please refer to our Product Catalog/Product Profiles,		
Product Guides and Internet Site.		

References:

Current Edition Merck Index 1)

Biological Industries(BI) Specifications 2)

Darling, D.C. and Morgan, S.J. <u>Animal Cells: Culture and Media</u>, New York: John Wiley & Sons, 1994 Lackie, J. M. <u>The Dictionary of Cell & Molecular Biology</u>, Academic Press: London, 200 3)

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