

Version: 1.0 Date:10/2009 Page 1 of 3 Pages

Product Profile

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Product Name:	DMEM without D-Glucose , without Sodium		
	Pyruvate, without - L-Glutamine		
Product Catalog Number	01-057-1		
Unit Size Availability:	(A)500ml ;(B)100ml		
Concentration:	1X		
Formulation:	Red-Colored Solution		
Specified Storage Conditions:	2-8°C		
Stability: (Under Specified Handling &	Please Refer to the Product Label		
Storage)			

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

Product Description:

DMEM, without D-Glucose, without Sodium Pyruvate, without L-Glutamine is a Dulbecco's modification of Eagle's medium (BME) that is considered one of the more common (e.g. MEM & RPMI) and less complex in contrast to enriched media like Ham's F-12 or CMRL among others which are utilized not only for more specialized cell types but also as the basis for some of the more unique Serum-Free Media formulations. DMEM contains concentrations of Amino Acids (AA's) and vitamins in addition to other ancillary constituents. The original DMEM formulation contains 1000mg/L of glucose and was first reported for culturing Mouse Embryonic Cells MEC's). A higher glucose level (4500mg/l) in some DMEM formulations has proven to be optimal for the cultivation of other cell types. This medium contains no L-Glutamine, neither D-Glucose nor Sodium Pyruvate that are usually part and parcel of many media formulations.

L-Glutamine

When used as a supplement, 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 a central and key participant in nitrogen metabolism. Although L-Glutamine supports the growth of cells with high energy demands and those that synthesize large quantities of nucleic acids and proteins, it is relatively unstable. L-Glutamine is simply a readily-available and viable alternative energy source for rapidly dividing cells as well as to cells that utilize glucose but in an inefficient manner. The resultant glucose-deficiency must offset this imbalance in order to meet the high energy demands of the cells. This is where the amino acids come into play and once deaminated, L-Glutamine is utilized as an essential energy source, segued into protein and participates in nucleic acid metabolism. Sometimes *L-Alanyl-L-Glutamine* is preferred over the regular L-Glutamine as it is a much more heat-stable dipeptide substitute for L-Glutamine.

Glucose

Glucose is an energy source (along with L-Glutamine) for most media. Traditional glucose levels in culture media usually range from 1-4.g g/l. From a general perspective, it may be said that cell line metabolic rate is directly proportional and thus correlates to the optimal glucose level. A cell line known to grow at a slow pace will grow in low or high glucose levels. However, a cell line with faster-growing cells requires higher glucose levels to maintain its metabolic rate and exposure to lower-than-optimal glucose levels in such characteristically fast-growing milieu may induce these types of cell lines to enter a lag phase.

Pyruvate

Pyruvate can serve several masters. It, not only, can be further oxidized to produce energy and CO₂, but also may be converted to lactate as it seques into the Krebs Citric Acid Cycle. However, in some cell lines, the Kreb's Cycle does not function normally and therefore their dependence on an alternative energy source such as Glutamine may be very high. Supplementation, when needed, with 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.

Sodium Pyruvate like Glutamine and Glucose are considered to be important constituent s of most media and are now recognized as an easily accessible additional carbohydrate energy source. Pyruvate, an anion of pyruvic acid, is the end product of glycolysis in which organisms break down glucose into lactic acid in the absence of molecular oxygen for the purpose of obtaining chemical energy. The glucose is converted to pyruvate with the production of Adenosine Triphosphate (ATP). Glycolysis is just one of several pathways used by different species to degrade glucose anaerobically. In the mitochondria of aerobic organisms, pyruvate is converted to Acetyl-CoA which in turn is completely oxidized to Carbon Dioxide (CO₂). Acetyl CoA is not only the initiator for the Krebs cycle; it is also formed during the metabolism of Fats, certain Amino Acids and also is utilized in the biosynthesis of a variety of larger molecules. The Krebs cycle doesn't consume energy; it produces it most efficiently. The cycle is fed by pyruvic acid from this anaerobic glycolysis pathway. Glycolysis releases energy and part of that energy goes to the conversion of ATP where it is stored. ATP provides the energy which drives cellular metabolic reactions and is considered the most important high-energy compounds in cells. Approximately 70% of the energy in the ATP comes from Carbohydrate Metabolism.

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Most common types of media consists 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 meeting their metabolic demands.

DMEM, without D-Glucose, without Sodium Pyruvate, without 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, and hormones) complement a typical basal or balanced salt solution medium or more complex media such as Iscove's Modification of DMEM.

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.

Sodium Bicarbonate

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 \sim 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 that plays a vital role in the success of the 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. *BI's* 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.

DMEM, without D-Glucose, without Sodium Pyruvate, without L-Glutamine contains numerous important basic constituents in a ready-touse formulation that includes a typical and wide variety of elements, among others:

- Amino Acids
- Glucose
- Inorganic Salts
- Vitamins
- Trace Elements

Some Predominant Characteristics of DMEM, without D-Glucose, without Sodium Pyruvate, without L-Glutamine, include:

- § Liquid Formulation
- § <u>Without</u> D- Glucose
- § <u>Without</u> Sodium Pyruvate
- § <u>Without</u> L-Glutamine
- § With Sodium Bicarbonate(NaHCO₃)
- § With Phenol Red(C₁₉H₁₃NaO₅S) as a pH indicator
- § More Uniform & Consistent Media Performance
- Sterile-Filtered(0.1μ),Cell-Culture and Endotoxin-Tested

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

DMEM, without D-Glucose, without Sodium Pyruvate, without 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, <u>deterioration of liquid media</u> 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.

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Instructions/Procedure:

- Take a bottle from the defined storage conditions at 2-8°C and read the label. 1)
- 2) Wipe the outside of the bottle with a disinfectant solution such as 70% ethanol.
- 3) Using aseptic/sterile technique under a laminar-flow culture hood, work according to established protocols.
- 4) Antibiotics may be added if desired.

Test	Specification
Cell-Culture:	Pass
Cell Line:	A-549
Endotoxins:	Test and Record
Osmolality:	300-334 mOsm/kg
pH :	7.0-7.5
Sterility	Test & Record

Auxiliary Products

Product Name	Catalog Number	Storage Temperature
DMEM Low Glucose 5X, without L-Glutamine, without Sodium	01-050-4	15-30°C
Bicarbonate		
DMEM with D- Glucose 4500mg/L, without Sodium Pyruvate, without	01-053-1	2-8°C
L-Glutamine, without Phenol Red		
DMEM with D- Glucose 4500mg/L, without Sodium Pyruvate, without	01-054-1	2-8°C
L-Glutamine, without L-Methionine		
DMEM with D- Glucose 4500mg/L, without Sodium Pyruvate, without	01-055-1	2-8°C
L-Glutamine		
DMEM 5X Conc., with D- Glucose 4500mg/L, without L-Glutamine,	01-055-4	Room Temperature(15-30°C)
without Sodium Pyruvate,		
DMEM High Glucose 2X, without L-Glutamine, with Sodium	01-055-9	2-8°C
Bicarbonate		
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	Room Temperature (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
DMEM with D- Glucose 1000mg/L, with Sodium Pyruvate 110mg/L,	06-1050-18-1	2-8°C
with Hepes(20mM)		
Note: For a list of Antibiotics, Serum or other Reagents and		
Supplements, please refer to our Product Catalog/Product Profiles/		
Product Guides and Internet Site.		

References:

- Biological Industries (BI) Specifications
 Darling, D.C. and Morgan S.J. <u>Animal Cells: Culture and Media</u>, John Wiley & Sons, New York, 1994
- O'Neil Maryadele et. al., The <u>Merck Index</u>, 14th Edition, Whitehouse Station, New Jersey, 2006
 Lackie, J. M. <u>The Dictionary of Cell & Molecular Biology</u>, Academic Press: London, 2007



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