

Technical Information

Leibovitz's L-15 Medium

With L-Glutamine Without Phenol red and Sodium bicarbonate

Product Code: AT1207

Application : Leibovitz's Medium was specifically designed to grow cells in a CO₂ free atmosphere. The standard sodium bicarbonate/CO₂ buffering system is replaced by combination of free basic amino acids, phosphate buffers and higher levels of galactose and sodium pyruvate. As a result, the medium does not require supplementation with sodium bicarbonate and can be used under conditions of free gaseous exchange with the atmosphere. The medium can be used to grow human tumor cells and embryonic cells and also established cell lines like HeLa and Hep-2. The medium is frequently used in diagnostic virology where tissue cell lines or strains need to be grown in closed systems. Leibovitz's medium obviates the need for frequent medium change.

AT1207 is Leibovitz's Medium with L-glutamine. It does not contain phenol red. Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

Composition**

Ingredients	mg/Litre
INORGANIC SALTS	
Calcium chloride dihydrate	185.000
Magnesium chloride hexahydrate	200.000
Magnesium sulphate anhydrous	97.720
Potassium chloride	400.000
Potassium phosphate monobasic	60.000
Sodium chloride	8000.000
Sodium phosphate dibasic anhydrous	190.120
AMINO ACIDS	
DL-Alpha alanine	450.000
Glycine	200.000
L-Arginine (free base)	500.000
L-Asparagine	250.000
L-Cysteine (free base)	120.000
L-Glutamine	300.000
L-Histidine (free base)	250.000
L-Isoleucine	250.000
L-Leucine	125.000
L-Lysine hydrochloride	94.000
L-Methionine	75.000
L-Phenylalanine	125.000

L-Serine	200.000
L-Threonine	300.000
L-Tryptophan	20.000
L-Tyrosine disodium salt	276.160
L-Valine	100.000
VITAMINS	
Choline chloride	1.000
D-Ca-Pantothenate	1.000
Folic acid	1.000
Nicotinamide	1.000
Pyridoxine hydrochloride	1.000
Riboflavin-5-phosphate sodium	0.100
Thiamine monophosphate	1.000
i-Inositol	2.000
OTHERS	
D-Galactose	900.000
Sodium pyruvate	550.000

Methodology

1. Suspend 14.0 gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
Note: Presence of slight haziness in the medium is due to inherent nature of some of the ingredients in the composition. However, this will not affect the performance of the medium.
2. Adjust the pH to 0.2-0.3 pH units below the desired pH using 1N HCl or 1N NaOH since the pH tends to rise during filtration make up the final volume to 1000ml with tissue culture grade water.
3. Sterilize the medium immediately by filtering through a sterile membrane filter with a porosity of 0.22 micron or less, using positive pressure rather than vacuum to minimize the loss of carbon dioxide.
4. Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into sterile containers.
5. Store liquid medium at 2-8°C and in dark till use.

Material required but not provided :

Tissue culture grade water (TCL1010)
 1N Hydrochloric acid (TCL1003)
 1N Sodium hydroxide (TCL1002)
 Foetal bovine serum (BA3112/BA12432)

Quality Control

Appearance

Off-white to Creamish white, homogenous powder

Solubility

Clear solution at 14.0gms/L.

pH without Sodium Bicarbonate

7.70-8.30

Osmolality without Sodium Bicarbonate

300.00 -340.00

Cultural Response

The growth promotion capacity of the medium is assessed qualitatively by analyzing the cells for the morphology and quantitatively by estimating the cell counts.

Endotoxin Content

NMT 5EU/ml

Storage and Shelf Life

1. All the powdered media and prepared liquid culture media should be stored at 2-8°C. Use before the expiry date. In spite of above recommended storage condition, certain powdered medium may show some signs of deterioration /degradation in certain instances. This can be indicated by change in colour, change in appearance and presence of particulate matter and haziness after dissolution.
2. Preparation of concentrated medium is not recommended since free base amino acids and salt complexes having low solubility may precipitate in concentrated medium.
3. pH and sodium bicarbonate concentration of the prepared medium are critical factors affecting cell growth. This is also influenced by amount of medium and volume of culture vessel used (surface to volume ratio). For example, in large bottles, such as Roux bottles pH tends to rise perceptibly as significant volume of carbon dioxide is released. Therefore, optimal conditions of pH, sodium bicarbonate concentration, surface to volume ratio must be determined for each cell type. We recommend stringent monitoring of pH. If needed, pH can be adjusted by using sterile 1N HCl or 1N NaOH or by bubbling in carbon dioxide.
4. If required, supplements can be added to the medium prior to or after filter sterilization observing sterility precautions. Shelf life of the medium will depend on the nature of supplement added to the medium.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conforms solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at CDH is true and accurate.
- **Central Drug House Pvt. Ltd.** reserves the right to make changes to specifications and information related to the products at any time.
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- Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.
- Do not use the products if it fails to meet specifications for identity and performance parameters.