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Technical Datasheet

Leibovitz's L-15 Medium

Without L- Glutamine and Sodium bicarbonate

Product Code: AT011A

Product Description :

Leibovitz's Medium was specifically designed to grow cells in a CO_2 free atmosphere. The standard sodium bicarbonate/CO_2 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 of frequent medium change.

AT011A is Leibovitz's Medium. It does not contain L-glutamine. Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

Composition :

Ingredients	mg/L
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	
L-Alanine	225.000
Glycine	200.000
L-Arginine (free base)	500.000
L-Asparagine monohydrate	284.070
L-Cysteine (free base)	120.000
L-Histidine (free base)	250.000
L-Isoleucine	125.000
L-Leucine	125.000
L-Lysine hydrochloride	93.700
L-Methionine	75.000
L-Phenylalanine	125.000
L-Serine	200.000
L-Threonine	300.000
L-Tryptophan	20.000
L-Tyrosine disodium salt dihydrate	430.000
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 salt	0.100
Thiamine monophosphate	1.000
i-Inositol	2.000
OTHERS	
D-Galactose	900.000
Phenol red sodium salt	10.000
Sodium pyruvate	550.000

Directions :

1. Suspend 13.7gms 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. Add 0.3gms of L-Glutamine powder (TC243) or 10.3ml of 200mM L-Glutamine solution (TCL012) for 1 litre of medium and stir until dissolved.
3. 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.
4. Make up the final volume to 1000ml with tissue culture grade water.
5. Sterilize the medium immediately by filtering through a sterile membrane filter with porosity of 0.22 micron or less,

using positive pressure rather than vacuum to minimize the loss of carbon dioxide.
6. Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into sterile containers.
7. Store liquid medium at 2-8ºC and in dark till use.

Material required but not provided :

Tissue culture grade water (TCL010)
L-Glutamine powder (TC243)
L-Glutamine solution 200mM (TCL012)
Sodium bicarbonate (TC230)
Sodium bicarbonate solution 7.5% (TCL013)
1N Hydrochloric acid (TCL003)
1N Sodium hydroxide (TCL002)
Foetal bovine serum (RM1112/RM10432)

Quality Control:

Appearance
White to light pink, homogenous powder

Solubility
Clear solution at 13.7 gms/L.

pH without Sodium Bicarbonate
8.00 -8.60

Osmolality without Sodium Bicarbonate(mOsm/Kg H_2O)
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 1EU/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.