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Product Specification

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Technical Information

William's Medium E

With L-Glutamine Without Sodium bicarbonate

Product Code: AT1125

Application:- William's Medium E was developed by Williams and Gunn for the isolation and long term maintenance of adult rat liver epithelial cells. The medium is a rich modification of William's medium D and was developed during studies performed to explore the possibilities of culturing adult liver cells on a long-term basis.

AT1125 is William's Medium with 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 / Litre
INORGANIC SALTS	
Calcium chloride dihydrate	265.000
Copper sulphate pentahydrate	0.0001
Ferric nitrate nonahydrate	0.0001
Magnesium sulphate anhydrous	97.700
Manganese chloride tetrahydrate	0.0001
Potassium chloride	400.000
Sodium chloride	6800.000
Sodium dihydrogen phosphate	122.000
Zinc sulphate heptahydrate	0.0002
AMINO ACIDS	
Glycine	50.000
L-Alanine	90.000
L-Arginine hydrochloride	60.300
L-Asparagine anhydrous	16.540
L-Aspartic acid	30.000
L-Cysteine dihydrochloride	64.070
L-Cystine	20.000
L-Glutamic acid	44.500
L-Glutamine	292.000
L-Histidine hydrochloride	18.580
L-Isoleucine	50.000
L-Leucine	75.000
L-Lysine hydrochloride	87.460
L-Methionine	15.000
L-Phenylalanine	25.000
L-Proline	30.000
L-Serine	10.000
L-Threonine	40.000
L-Tryptophan	10.000
L-Tyrosine Disodium Salt	43.500
L-Valine	50.000

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VITAMINS	
Ascorbic acid sodium salt	2.270
Calciferol	0.100
Choline chloride	15.000
D-Biotin	0.500
D-Ca-Pantothenate hemicalcium	1.000
Folic acid	1.000
Menadione sodium bisulphite	0.010
Niacinamide	1.000
Pyridoxal hydrochloride	1.000
Retinol acetate	0.100
Riboflavin	0.100
Thiamine hydrochloride	1.000
Vitamin B12	0.200
myo-Inositol	2.000
OTHERS	
D-Glucose	2000.000
Glutathione reduced	0.050
Methyl linoleate	0.030
Phenol red	10.700
Pyruvate acid sodium salt	25.000

Methodology

1. Suspend 10.9 gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 2.2gms of sodium bicarbonate powder (TC1230) or 29.3ml of 7.5% sodium bicarbonate solution (TCL1013) for 1litre 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 a 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 2-8 ºC and in dark till use

Material required but not provided

Tissue culture grade water (TCL1010)
Sodium bicarbonate (TC1230)
Sodium bicarbonate solution, 7.5% (TCL1013)
1N Hydrochloric acid (TCL1003)
1N Sodium hydroxide (TCL1002)
Foetal bovine serum (BA3112/BA30432)

Quality Control

Appearance
Off-white to Creamish white, homogenous powder .

Solubility
Clear solution at 10.9gms/L

pH without Sodium Bicarbonate
3.70-4.30

pH with Sodium Bicarbonate
7.20-7.80

Osmolality without Sodium Bicarbonate
240.00-280.00

Osmolality with Sodium Bicarbonate
290.00-330.00

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 and comparing it with a control medium through minimum three subcultures.

Endotoxin Content
NMT 5EU/ml

Storage and Shelf Life

1. All the powdered media and prepared liquid culture media should be stored at 2-8ºC. 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.
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- Do not use the products if it fails to meet specificatons for identity and performens parameters.