CDH

Product Specification

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Technical Information

Medium 199
With Earle's salts and 25mM HEPES buffer
Without L- Glutamine and Sodium bicarbonate

Product Code: AT1094A

Application: - Medium 199 was the first nutritionally defined medium developed by Morgan, Morton, and Parker in 1950. This complex medium was formulated specifically for nutritional studies on primary chick embryo fibroblasts in the absence of any additives. It was observed that explanted tissue could survive in Medium 199 without serum but long term cultivation of cells required supplementation of the medium with serum.

Medium 199 is formulated with either Hank's salts or Earle's salts. The medium when supplemented with serumcan be used for growth of a wide variety of cells. Medium 199 is presently used for the maintenance of non-transformed cells, vaccine and virus production andprimary explants of epithelial cells.

AT1094 is Medium 199 with Earle's salts, 25mM HEPES buffer and L-glutamine. HEPES, a zwitterionic buffer having a pKa of 7.3 at 37ºC prevents the initial rise in pH that tends to occur at the initiation of a culture and increases the buffering capacity of the medium. 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
Ferric nitrate nonahydrate	0.720
Magnesium sulphate anhydrous	97.720
Potassium chloride	400.000
Sodium acetate anhydrous	50.000
Sodium chloride	6800.00.
Sodium dihydrogen phosphate anhydrous	122.000
AMINO ACIDS	
Glycine	50.000
L-Alanine	25.000
L-Arginine hydrochloride	70.000
L-Aspartic acid	30.000
L-Cysteine hydrochloride monohydrate	0.100
L-Cystine dihydrochloride	26.000
L-Glutamic acid	67.000
L-Histidine hydrochloride monohydrate	22.000
L-Hydroxyproline	10.000
L-Isoleucine	20.000
L-Leucine	60.000
L-Lysine hydrochloride	70.000
L-Methionine	15.000
L-Phenylalanine	25.000
L-Proline	40.000

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L-Serine	25.000
L-Threonine	30.000
L-Tryptophan	10.000
L-Tyrosine Disodium Salt	57.660
L-Valine	25.000
VITAMINS	
Ascorbic acid	0.050
Calciferol	0.100
Choline chloride	0.500
D-Biotin	0.010
D-Ca-Pantothenate	0.010
DL-Tocopherol phosphate disodium salt	0.010
Folic acid	0.010
Menadione	0.010
Nicotinamide	0.025
Nicotinic acid	0.025
Pyridoxal hydrochloride	0.025
Pyridoxine hydrochloride	0.025
Retinol Acetate	0.140
Riboflavin	0.010
Thiamine hydrochloride	0.010
i-Inositol	0.050
p-Amino benzoic acid (PABA)	0.050
OTHERS	
Adenine sulphate	10.000
Adenosine monophosphate	0.200
Adenosine triphosphate	1.000
Cholesterol	0.200
Deoxyribose	0.500
Glucose	1000.00
Glutathione reduced	0.050
Guanine hydrochloride	0.300
HEPES Buffer	5958.00
Hypoxanthine	0.354
Phenol red	15.000
Polysorbate 80	4.900
Ribose	0.500
Thymine	0.300
Uracil	0.300
Xanthine	0.344

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Methodology

1. Suspend 15.5gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 0.1gms of L-glutamine (TC1230) or 3.42ml of 200mML-glutamine (TCL1012) and 2.2gms of sodium bicarbonate powder (TC1243) or 29.3ml of 7.5% sodium bicarbonatesolution (TCL1013) 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 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°C and in dark till use.

Material required but not provided

Tissue culture grade water (TCL1010)
Sodium bicarbonate (TC1230)
Sodium bicarbonate solution, 7.5% (TCL1013)
L-Glutamine powder (TC1243)
L-Glutamine solution 200mM (TCL1012)
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 15.5 gms/L

pH without Sodium Bicarbonate
4.80-5.40

pH with Sodium Bicarbonate
6.60-7.20

Osmolality without Sodium Bicarbonate
265.00-305.00

Osmolality with Sodium Bicarbonate
310.00-350.00

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.