Technical Data Sheet

Medium 199 (powder) with Hanks' salts, with L-Glutamine, w/o NaHCO3

for cell biology

Order number: **2491**

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 serum can 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 and primary explants of epithelial cells.

Composition of 2491 powder medium

Ingredients	concentration [mg/l]
Inorganic salts	
Calcium chloride dihydrate	185.000
Ferric nitrate nonahydrate	0.720
Magnesium sulphate anhydrous	97.670
Potassium chloride	400.000
Potassium phosphate, monobasic	60.000
Sodium acetate anhydrous	50.000
Sodium chloride	7500.000
Sodium phosphate anhydrous	47.860
Amino acids	
Glycine	50.000
L-Alanine	25.000
L-Arginine hydrochloride	70.000
L-Aspartic acid	30.000
L-Cysteine hydrochloride monohydrate	0.110
L-Cystine dihydrochloride	26.000
L-Glutamic acid	75.000
L-Glutamine	100.000
L-Histidine hydrochloride monohydrate	21.800
L-Hydroxyproline	10.000
L-Isoleucine	40.000
L-Leucine	60.000
L-Lysine hydrochloride	70.000
L-Methionine	15.000
L-Phenylalanine	25.000

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Ingredients	concentration [mg/l]
L-Proline	40.000
L-Serine	25.000
L-Threonine	30.000
L-Tryptophan	10.000
L-Tyrosine disodium salt	40.000
L-Valine	25.000
Vitamins	
Ascorbic acid	0.056
Calciferol	0.100
Choline chloride	0.500
D-Biotin	0.010
D-Pantothenic Acid	0.010
DL-Tocopherol phosphate disodium salt	0.010
Folic acid	0.010
Menadione	0.016
Niacinamide	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
myo-Inositol	0.050
p-Amino benzoic acid (PABA)	0.050
others	
Adenine sulphate	10.000
Adenosine monophosphate	0.238
Adenosine triphosphate	1.000
Cholesterol	0.200
Deoxyribose	0.500
Glucose	1000.000
Glutathione reduced	0.050
Guanine hydrochloride	0.300
Hypoxanthine	0.400
Phenol red	20.000
Tween 80	20.000
Ribose	0.500
Thymine	0.300
Uracil	0.300
Xanthine sodium salt	0.344

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Instruction for use

1. Suspend 10.6 g of the powder medium in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 0.35 g of sodium bicarbonate powder 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 1000 ml 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.

Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

Recommendations

- Preparation of concentrated medium is not recommended since free base amino acids and salt complexes having low solubility may precipitate in concentrated medium.
- 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.
- If required, supplements can be added to the medium prior to or after filter sterilization observing sterility precautions.

Storage

The powdered medium and the freshly prepared liquid culture medium should be stored at 2-8°C. Use before the expiry date. Shelf life of the liquid medium will depend on the nature of the supplement added to the medium.

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