ProductInformation

MEDIUM 199 MODIFIED
With Hanks' Salts, Without L-Glutamine,
Phenol Red and Sodium Bicarbonate

Product Number **M3274**
Storage Temperature 2-8°C

Product Description

Many early tissue culture media were predominantly formulated from animal products and/or tissue extracts. In 1950, Morgan and his coworkers reported their efforts to produce a totally defined nutritional source for cell cultures. Their experiments, conducted with various combinations of vitamins, amino acids and other factors revealed that growth of explanted tissue could be measured in what has become known as Medium 199. However, it was found that long-term cultivation of cells required addition of a serum supplement to the culture fluid. When properly supplemented, Medium 199 has broad species applicability, particularly for cultivation of non-transformed cells. It is widely used in virology, vaccine production and in vitro cultivation of primary explants of mouse pancreatic epithelial and rat lens tissues.

MEDIUM 199 MODIFIED, Product No. M3274 is one of the cell culture media available from Sigma. The selection of a nutrient medium is strongly influenced by 1] type of cell, 2] type of culture [monolayer, suspension, clonal] and 3] degree of chemical definition necessary. It is important to review the literature for recommendations concerning medium, supplementation and physiological parameters required for a specific cell line.

Components	g/L
Calcium Chloride•2 H_2O	0.185
Ferric Nitrate•9 H_2O	0.00072
Magnesium Sulfate (anhydrous)	0.09767
Potassium Chloride	0.4
Potassium Phosphate Monobasic	0.06
Sodium Acetate (anhydrous)	0.05
Sodium Chloride	8.0
Sodium Phosphate Dibasic (anhydrous)	0.04788
DL-Alanine	0.05
L-Arginine•HCl	0.07
DL-Aspartic Acid	0.06
L-Cysteine•HCl• H_2O	0.00011
L-Cystine•2HCl	0.026
DL-Glutamic Acid	0.1336
Glycine	0.05
L-Histidine•HCl• H_2O	0.02188
Trans-4-Hydroxy-L-Proline	0.01
DL-Isoleucine	0.04
DL-Leucine	0.12
L-Lysine•HCl	0.07
DL-Methionine	0.03
DL-Phenylalanine	0.05
L-Proline	0.04
DL-Serine	0.05
DL-Threonine	0.06
DL-Tryptophan	0.02
L-Tyrosine 2Na•2 H_2O	0.05766
DL-Valine	0.05
Ascorbic Acid•Na	0.0000566
D-Biotin	0.00001
Ergocalciferol	0.0001
Choline Chloride	0.0005
Folic Acid	0.00001
Menadione (sodium bisulfite)	0.000016
myo-Inositol	0.00005
Niacinamide	0.000025
Nicotinic Acid	0.000025
p-Aminobenzoic Acid	0.00005
D-Pantothenic Acid (hemicalcium)	0.00001
Pyridoxal•HCl	0.000025
Pyridoxine•HCl	0.000025
Retinol Acetate	0.00014
Riboflavin	0.00001
DL-α-Tocopherol Phosphate•2Na	0.00001
Thiamine•HCl	0.00001
Adenine Hemisulfate	0.01
Adenosine-5'-Triphosphate•2Na	0.001
Adenosine-5'-Monophosphate•Na	0.0002385
Cholesterol	0.0002
2-Deoxy-D-Ribose	0.0005
Glucose	1.0
Glutathione (reduced)	0.00005
Guanine•HCl	0.0003
Hypoxanthine	0.0003
Polyoxyethylenesorbitan Monooleate (Tween 80)	0.02
Ribose	0.0005
Thymine	0.0003
Uracil	0.0003
Xanthine•Na	0.000344

Precautions and Disclaimer

REAGENT
For In Vitro Diagnostic Use

Preparation Instructions

Powdered media are extremely hygroscopic and should be protected from atmospheric moisture. The entire contents of each package should be used immediately after opening. Preparing a concentrated solution of medium is not recommended as precipitates may form.

Supplements can be added prior to filtration or introduced aseptically to sterile medium. The nature of the supplement may affect storage conditions and shelf life of the medium.

1. Measure out 90% of final required volume of water. Water temperature should be 15-20°C.
2. While gently stirring the water, add the powdered medium. Stir until dissolved. Do NOT heat.
3. Rinse original package with a small amount of water to remove all traces of powder. Add to solution in step 2.
4. To the solution in step 3, add 0.35 g sodium bicarbonate or 4.7 ml of sodium bicarbonate solution [7.5%w/v] for each liter of final volume of medium being prepared. Stir until dissolved.
5. While stirring, adjust the pH of the medium to 0.1-0.3 pH units below the desired pH since it may rise during filtration. The use of 1N HCl or 1N NaOH is recommended.
6. Add additional water to bring the solution to final volume.
7. Sterilize immediately by filtration using a membrane with a porosity of 0.22 microns.
8. Aseptically dispense medium into sterile container.

Storage/Stability
Store the dry powdered medium at 2-8°C under dry conditions and liquid medium at 2-8°C in the dark. Deterioration of the powdered medium may be recognized by any or all of the following: [1] color change, [2] granulation/clumping, [3] insolubility. Deterioration of the liquid medium may be recognized by any or all of the following: [1] pH change, [2] precipitate or particulate matter throughout the solution, [3] cloudy appearance [4] color change. The nature of supplements added may affect storage conditions and shelf life of the medium. Product label bears expiration date.

Procedure
Materials Required but Not Provided
Water for tissue culture use [W3500]
Sodium Bicarbonate [S5761] or
Sodium Bicarbonate Solution, 7.5% [S8761]
1N Hydrochloric Acid [H9892]
1N Sodium Hydroxide [S2770]
Medium additives as required

Product Profile

Appearance	off-white powder
Moisture content	≤ 2.0%
Solubility	clear solution at 1x concentration
pH at RT [without sodium bicarbonate]	4.5 ± 0.3
pH at RT [with sodium bicarbonate]	6.6 ± 0.3
Osmolality [without sodium bicarbonate]	287 mOsm/kg H_2O ± 5%
Osmolality [with sodium bicarbonate]	303 mOsm/kg H_2O ± 5%
Endotoxin	≤1.0 EU/ml at 1x
Amino Acid Analysis by HPLC	Analysis has confirmed that amino acids are present at concentrations consistent with the formula.
Key Element Analysis by ICAP	Analysis has confirmed that key elements are present at concentrations consistent with the formula.

Biological Performance Characteristics
Biological performance is assessed using an appropriate cell line(s). Growth studies are carried through 2 subculture generations. Cells are counted and growth is plotted as a logarithmic function of time in culture. Seeding efficiencies, doubling time, and final cell densities are determined. During the testing period cultures are examined microscopically for atypical morphology and evidence of cytotoxicity. Test results are available upon request.

References

1. Morgan, J.F., Morton, H.J. and Parker, R.C. (1950) The Nutrition of Animal Cells in Tissue Culture. I. Initial Studies on a Synthetic Medium. Proc. Soc. Exp. Biol. Med. 73, 1-8.
2. Morgan, J.F., Campbell, E. and Morton, H.J. (1955) The Nutrition of Animal Tissues Cultivated In Vitro. I. A Survey of Natural Materials as Supplements to Synthetic Medium. J.N.C.I. 16:2, 557-567.

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