SAFC Biosciences™
Accelerate Success™

Product Information

Medium 199 Modified

with Earle's Balanced Salts, with 0.68 mM L-glutamine, without sodium bicarbonate

CATALOG NO. 56312C

Description

In 1950, Morgan, Morton and Parker described one of the first totally defined media that did not depend largely on animal products or extracts as nutritive sources. This medium supported the growth of primary chick embryo heart cells and has since become known as Medium 199 (M199). Its current use is with the addition of serum in virus and vaccine production, and in the culture of many non-transformed cells. It is also used in combination with less complex media.

M199 Modified with Earle's Balanced Salts is designed for use with cells maintained in a CO_2 environment. This modified M199 formulation differs from the original formulation by containing all L-amino acids. The original formulation contained 10 DL-amino acids, which are less biologically active on a per-gram basis than the corresponding L-amino acids.

Formulation

Component (all components measured in mg/L)	
INORGANIC SALTS	
Calcium chloride anhydrous	200.000
Ferric nitrate nonahydrate	0.720
Magnesium sulfate anhydrous	97.670
Potassium chloride	400.000
Sodium acetate anhydrous	50.000
Sodium chloride	6800.000
Sodium phosphate monobasic monohydrate	140.000
VITAMINS	
Ascorbic acid	0.050
Biotin	0.010
D-calcium pantothenate	0.010
Choline chloride	0.500
Ergocalciferol	0.100
Folic acid	0.010
i-inositol	0.050
Menadione	0.010
Niacin	0.025
Niacinamide	0.025
PABA	0.050
Pyridoxal HCl	0.025
Pyridoxine HCl	0.025
Riboflavin	0.010
Thiamine HCl	0.010
DL-α-tocopherol phosphate 2Na	0.010
Vitamin A acetate	0.140

Component, continued (all components measured in mg/L)	
AMINO ACIDS	
L-alanine	25.000
L-arginine HCl	70.000
L-aspartic acid	30.000
L-cysteine HCl monohydrate	0.110
L-cystine 2HCl	26.000
L-glutamic acid	67.000
L-glutamine	100.000
Glycine	50.000
L-histidine HCl monohydrate	21.880
Hydroxy L-proline	10.000
L-isoleucine	20.000
L-leucine	60.000
L-lysine HCl	70.000
L-methionine	15.000
L-phenylalanine	25.000
L-proline	40.000
L-serine	25.000
L-threonine	30.000
L-tryptophan	10.000
L-tyrosine 2Na dihydrate	57.660
L-valine	25.000
OTHER	
Adenine sulfate dihydrate	10.980
AMP monohydrate	0.200
ATP 2Na trihydrate	1.098
Cholesterol (synthetic)	0.200
2-Deoxy-d-ribose	0.500
Dextrose anhydrous	1000.000
L-glutathione reduced	0.050
Guanine HCl monohydrate	0.300
Hypoxanthine sodium salt	0.354
Phenol red sodium salt	21.240
D-Ribose	0.500
Thymine	0.300
Tween™ 80	20.000
Uracil	0.300
Xanthine sodium salt	0.344
ADD: Sodium bicarbonate	2200.000
Grams of powder per liter	9.523

Precautions

Use aseptic technique when handling or supplementing this medium after filtration. This product is for further manufacturing use. THIS PRODUCT IS NOT INTENDED FOR HUMAN OR THERAPEUTIC USE.

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Storage

Store dry powder medium at 2 to 8 C. Do not use after the expiration date. Store hydrated medium protected from light at 2 to 8 C.

Indications of Deterioration

Dry powder medium should be free flowing. Do not use if powder is caked. Prepared medium should be clear of particulates and flocculent material. Do not use if liquid medium is cloudy or contains precipitate. Other evidence of deterioration may include color change or degradation of physical or performance characteristics.

Preparation Instructions

1. Measure 80 - 90% of the final volume of cell culture grade water (Catalog No. 59900C) into an appropriate size mixing vessel. Water temperature should be 15 to 30 C. Do not heat water.
2. Add the dry powder medium to the water. Rinse the original package with a small amount of cell culture grade water to remove all traces of powder and add to the solution. Mix until completely dissolved.
3. For each liter being prepared, add 2.20 g/L of sodium bicarbonate (Catalog No. 90421C) or 29.3 mL/L of sodium bicarbonate solution 7.5% (Catalog No. 59221C). Mix until completely dissolved.
4. While stirring the solution in Step 3, adjust the pH to 6.9 - 7.1 using NaOH 1N (Catalog No. 59223C) or HCl 1N. The pH of bicarbonate buffered solutions usually rises 0.1 - 0.2 units during filtration.
5. Add cell culture grade water to the solution in Step 4 to bring it to the final volume. Keep the vessel closed until the solution is filtered to avoid fluctuations in pH.
6. Sterilize the solution using a membrane filter with a pore size of 0.22 µm or less. A peristaltic pump or an inert gas such as nitrogen can be used to provide positive pressure at 3 - 15 psi. Do not use CO_2 gas.
7. Sterile solutions should be dispensed aseptically into sterile containers. Store protected from light at 2 to 8 C.
8. Supplements, such as antibiotics, can be added to the sterilized solution using aseptic technique. Storage conditions and shelf life of the supplemented product may be affected by the nature of the supplements. Sterile serum should not be refiltered before or after being added to sterile medium because growth promoting capacity may be reduced upon refiltration.

   NOTE: Dry powder medium is extremely hygroscopic and must be protected from atmospheric moisture. We recommend that the entire contents of each package be used immediately after opening.

Characteristics

Appearance
Off-white free-flowing powder

Bioburden
≤ 100 CFU/100 mL

Endotoxin
≤ 1.0 EU/mL

Osmolality (as supplied)
Refer to Certificate of Analysis for lot-specific value

pH (as supplied)
Refer to Certificate of Analysis for lot-specific value

References

1. Morgan, J. F., Morton, H. J., Parker, R. C., *PSEBM* (1950) 73:1.
2. Morgan, J. F., Campbell, M. E., Morton, H. J., *JNCI* (1955) 16:557.
3. Rutzky, L. P. and Pumper, R. W., *In Vitrol* (1974) 9:468.
4. Morton, H. J. and Toinai, S., *TCA Manual* (1978) 4:729.
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6. Bolton, W. E., Terrell, S. P., et al, *Tissue Culture Methods* (1982) 7:39.

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