

Medium 199 (1X), Liquid

With Hanks' salts
With L-glutamine
With sodium bicarbonate

Catalog Number **LM 006-06** (without HEPES)
LM 006-07 (with 25mM HEPES)

Storage Temperature 2~8°C

Product Description

Medium 199 contains various combinations of vitamins, amino acids and other factors. It has broad species applicability, particularly for cultivation of non-transformed cells. Morgan and his coworkers reported their efforts to produce a totally defined nutritional source, instead of serum, for cell cultures. However, it was found that long-term cultivation of cells required addition of a serum supplement to the culture. It is widely used in virology, vaccine production and in vitro cultivation of primary explants of mouse pancreatic epithelial and rat lens tissues. HEPES buffer provides more effective buffering in the optimum pH range of 7.2~7.4.

LM 006-06 is based on the Hanks' balanced salts, and contains 100 mg/L L-glutamine and 2200 mg/L sodium bicarbonate, but no HEPES. Add 25 ml of HEPES buffer (**BB 001-01**, 1 M) per 1 L of medium, if desired. **LM 006-07** is based on the Hanks' balanced salts, and contains 100 mg/L L-glutamine, 25 mM HEPES, and 2200 mg/L sodium bicarbonate. The selection of a nutrient medium is strongly influenced by (1) type of cell, (2) type of culture (monolayer, suspension, or 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.

Storage/Stability

The liquid medium should be stored at 2~8°C in the dark. Deterioration of the liquid medium may be recognized by (1) precipitate or particulate matter throughout the solution, (2) cloudy appearance, (3) color change, and/or (4) pH change. The nature of supplements added may affect storage conditions and shelf life of the medium. Product label bears expiration date.

Biological Performance Characteristics

The growth-promoting capacities of Medium 199 are tested in a liquid medium using an appropriate cell line(s). Growth rates are examined through three subculture generations and compared with parallel cultures grown in standardized control medium. Cells are counted and growth is plotted as a logarithmic function of time in culture, and seeding efficiency, doubling time, and the final cell density are determined. During the testing period cultures are examined microscopically for a typical morphology and evidence of cytotoxicity.

Product Profile	LM 006-06	LM 006-07
Appearance	Red translucent solution	Red translucent solution
pH at RT	7.0 ~ 7.6	7.0 ~ 7.6
Osmolality	274 ~ 303 mOsm/kg H ₂ O	318 ~ 350 mOsm/kg H ₂ O
Endotoxin	≤ 1.0 EU/ml	≤ 1.0 EU/ml
Sterility	Sterilized by 0.2 μm filtration system. Sterility tests are performed in accordance with protocols described in USP.	

	Salts	L-glutamine	HEPES	Phenol red
LM 006-01	Earle's	○	-	○
LM 006-02	Earle's	-	-	○
LM 006-03	Earle's	-	○	○
LM 006-04	Earle's	○	○	○
LM 006-05	Earle's	○	○	-
LM 006-06	Hanks'	○	-	○
LM 006-07	Hanks'	○	○	○

Precautions

For *In Vitro* Use Only

Components	LM 006-06	LM 006-07
CaCl ₂ (anhydrous)	140.00	140.00
Fe(NO ₃) ₃ ·9H ₂ O	0.72	0.72
KCl	400.00	400.00
KH ₂ PO ₄	60.00	60.00
MgSO ₄ (anhydrous)	98.00	98.00
NaCl	8000.00	8000.00
NaHCO ₃	350.00	350.00
NaH ₂ PO ₄ ·H ₂ O	-	-
Na ₂ HPO ₄ ·7H ₂ O	90.00	90.00
Adenine Sulfate	10.00	10.00
Adenosine-5-triphosphate	1.00	1.00
Adenosine-5-phosphate	0.20	0.20
Chloesterol	0.20	0.20
2-deoxy-D-ribose	0.50	0.50
D-Glucose	1000.00	1000.00
Glutathione (reduced)	0.05	0.05
Guanine-HCl	0.30	0.30
HEPES	-	5958.00
Hypoxanthine-Na	0.40	0.40
Phenol Red	20.00	20.00
Ribose	0.50	0.50
Sodium Acetate	50.00	50.00
Thymine	0.30	0.30
Tween 80	20.00	20.00
Uracil	0.30	0.30
Xanthine-Na	0.340	0.340
L-Alanine	25.00	25.00
L-Arginine-HCl	70.00	70.00
L-Aspartic Acid	30.00	30.00
L-Cystein-HCl·H ₂ O	0.10	0.10
L-Cysteine-2HCl	26.00	26.00
Glycine	50.00	50.00
L-Glutamic Acid	75.00	75.00
L-Glutamine	100.00	100.00
L-Histidine-HCl·H ₂ O	22.00	22.00
L-Hydroxyproline	10.00	10.00
L-Isoleucine	40.00	40.00
L-Leucine	60.00	60.00
L-Lysine-HCl	70.00	70.00
L-Methionine	15.00	15.00
L-Phenylalanine	25.00	25.00
L-Proline	40.00	40.00
L-Serine	25.00	25.00
L-Threonine	30.00	30.00
L-Tryptophan	10.00	10.00
L-Tyrosine-2Na·2H ₂ O	58.00	58.00
L-Valine	25.00	25.00
Ascorbic Acid	0.050	0.050
α-Tocopherol Phosphate (sodium salt)	0.01	0.01
Biotin	0.01	0.01
Calciferol	0.10	0.10
D-Ca Pantothenate	0.01	0.01
Choline Chloride	0.50	0.50
Folic Acid	0.01	0.01
i-Inositol	0.05	0.05
Menadione (sodium bisulfite)	0.01	0.01
Niacin	0.025	0.025
Niacinamide	0.025	0.025
Para-aminobenzoic Acid	0.05	0.05
Pyridoxal-HCl	0.025	0.025
Pyridoxine-HCl	0.025	0.025
Riboflavin	0.01	0.01
Thiamine-HCl	0.01	0.01
Vitamin A (acetate)	0.14	0.14

References

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.
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.
Morton, H.J. (1970). A Survey of Commercially Available Tissue Culture Media. In Vitro. 6, 89-108.
Rutzky, L.P. and Pumper, R.W., (1974). Supplement to a Survey of Commercially Available Tissue Culture Media (1970). In Vitro. 9, 468-469.

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제품설명

Medium 199에는 매우 다양한 종류의 비타민, 아미노산, 그리고 그 외의 여러 인자들이 포함되어 있다. Medium 199은 매우 다양한 종류의 세포 배양에 적용할 수 있으며 특히 형질전환 되지 않은 세포 배양에 적합하다. 1950년대 Morgan 등에 의해 무혈청 배지로 개발되었으나 장기간 배양하기 위해서는 혈청을 첨가하는 것이 좋다는 결과가 보고되기도 하였다. 현재 Medium 199은 바이러스 연구, 백신 생산, 그리고 mouse의 체장 상피조직과 rat의 수정체 조직의 초대 배양(primary explants) 등에 널리 사용된다. 또한 HEPES의 첨가로 인해 최적 pH인 7.3 근방에서 더욱 효과적인 완충작용을 나타낸다.

LM 006-06은 Hanks' balanced salts를 기본조성으로 하고, 100 mg/L의 L-glutamine과 2200 mg/L의 sodium bicarbonate이 포함되어 있으나 HEPES는 포함되어 있지 않다. 따라서 원하는 경우 배지 1 L 당 25 ml의 HEPES buffer (**BB 001-01**, 1 M)을 첨가하면 25 mM의 HEPES가 첨가된 배지로 사용할 수도 있다. **LM 006-07**는 Hanks' balanced salts를 기본조성으로 하고, 100 mg/L의 L-glutamine, 25 mM의 HEPES와 2200 mg/L의 sodium bicarbonate이 포함되어 있다. 적절한 배양액을 선택하기 위해서는 (1) 배양할 세포 종류, (2) 배양방법 (monolayer, suspension, or clonal), 그리고 (3) 필수 성분 포함 여부 등을 고려해야 한다. 또한 참고문헌을 기초로 하여 배양액에 혈청, 첨가물, 그리고 기타 물리적 조건 등을 최적화함으로써 배양하고자 하는 세포의 성장 및 목적 산물의 생산을 최적화할 수 있다.

보관 및 안정성

액상 배지는 차광하여 2~8°C에서 보관하여야 한다. 액상 배지의 변성은 (1) 침전물 또는 부유물, (2) 용액의 탁해짐, (3) 색의 변화, 그리고 (4) pH의 변화 등으로 나타날 수 있다. 추가로 첨가하는 첨가제의 성질에 의해 보관조건 및 배지의 유효기간이 바뀔 수 있다. 유효기간은 제품 라벨에 표시되어 있다.

생물학적 특성

Medium 199의 세포 증식 능력은 액상 배지에 적합한 세포주를 배양하면서 시험한다. 성장 속도는 세 번의 계대 배양을 통하여 측정하고 표준품에서 배양한 것과 비교한다. 시간에 따른 세포수의 변화를 측정하고 seeding efficiency, doubling time, 그리고 최종 세포농도를 결정한다. 시험을 하면서 현미경으로 세포의 형태 변화와 cytotoxicity의 현상이 나타나는지 관찰한다.

Product Profile	LM 006-06	LM 006-07
Appearance	Red translucent solution	Red translucent solution
pH at RT	7.0 ~ 7.6	7.0 ~ 7.6
Osmolality	274 ~ 303 mOsm/kg H ₂ O	318 ~ 350 mOsm/kg H ₂ O
Endotoxin	≤ 1.0 EU/ml	≤ 1.0 EU/ml
Sterility	Sterilized by 0.2 μm filtration system. Sterility tests are performed in accordance with protocols described in USP.	

	Salts	L-glutamine	HEPES	Phenol red
LM 006-01	Earle's	O	-	O
LM 006-02	Earle's	-	-	O
LM 006-03	Earle's	-	O	O
LM 006-04	Earle's	O	O	O
LM 006-05	Earle's	O	O	-
LM 006-06	Hanks'	O	-	O
LM 006-07	Hanks'	O	O	O

주의
For In Vitro Use Only

Components	LM 006-06	LM 006-07
CaCl ₂ (anhydrous)	140.00	140.00
Fe(NO ₃) ₃ ·9H ₂ O	0.72	0.72
KCl	400.00	400.00
KH ₂ PO ₄	60.00	60.00
MgSO ₄ (anhydrous)	98.00	98.00
NaCl	8000.00	8000.00
NaHCO ₃	350.00	350.00
NaH ₂ PO ₄ ·H ₂ O	-	-
Na ₂ HPO ₄ ·7H ₂ O	90.00	90.00
Adenine Sulfate	10.00	10.00
Adenosine-5-triphosphate	1.00	1.00
Adenosine-5-phosphate	0.20	0.20
Chloesterol	0.20	0.20
2-deoxy-D-ribose	0.50	0.50
D-Glucose	1000.00	1000.00
Glutathione (reduced)	0.05	0.05
Guanine-HCl	0.30	0.30
HEPES	-	5958.00
Hypoxanthine-Na	0.40	0.40
Phenol Red	20.00	20.00
Ribose	0.50	0.50
Sodium Acetate	50.00	50.00
Thymine	0.30	0.30
Tween 80	20.00	20.00
Uracil	0.30	0.30
Xanthine-Na	0.340	0.340
L-Alanine	25.00	25.00
L-Arginine-HCl	70.00	70.00
L-Aspartic Acid	30.00	30.00
L-Cystein-HCl·H ₂ O	0.10	0.10
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L-Hydroxyproline	10.00	10.00
L-Isoleucine	40.00	40.00
L-Leucine	60.00	60.00
L-Lysine-HCl	70.00	70.00
L-Methionine	15.00	15.00
L-Phenylalanine	25.00	25.00
L-Proline	40.00	40.00
L-Serine	25.00	25.00
L-Threonine	30.00	30.00
L-Tryptophan	10.00	10.00
L-Tyrosine-2Na·2H ₂ O	58.00	58.00
L-Valine	25.00	25.00
Ascorbic Acid	0.050	0.050
α-Tocopherol Phosphate (sodium salt)	0.01	0.01
Biotin	0.01	0.01
Calciferol	0.10	0.10
D-Ca Pantothenate	0.01	0.01
Choline Chloride	0.50	0.50
Folic Acid	0.01	0.01
i-Inositol	0.05	0.05
Menadione (sodium bisulfite)	0.01	0.01
Niacin	0.025	0.025
Niacinamide	0.025	0.025
Para-aminobenzoic Acid	0.05	0.05
Pyridoxal-HCl	0.025	0.025
Pyridoxine-HCl	0.025	0.025
Riboflavin	0.01	0.01
Thiamine-HCl	0.01	0.01
Vitamin A (acetate)	0.14	0.14

참고문헌

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