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ProductInformation

MCDB 302 MEDIUM COMPLETE WITH TRACE ELEMENTS
With L-Glutamine, Without Nucleosides and Sodium Bicarbonate

Product Number **M 2021**
Storage Temperature 2-8 °C

Product Description
MCDB media were designed for the low-protein or serum-free growth of specific cell types using hormones,growth factors, trace elements or low levels of dialyzed fetal bovine serum protein (FBSP). Each MCDB medium was formulated (qualitatively and quantitatively) to provide a defined and optimally balanced nutritional environment that selectively promoted growth of a specific cell type. MCDB 105 and 110 are modifications of MCDB 104 medium, optimized for long-term survival and rapid clonal growth of human diploid fibroblast-like cells (WI-38, MRC-5, IMR-90) and of low-passage human foreskin fibroblasts using FBSP or hormone and growth factor supplements. MCDB 151, 201 and 302 are modifications of Ham's nutrient mixture F-12, designed for the growth of human keratinocytes, clonal growth of chicken embryo fibroblasts and chinese hamster ovary (CHO) cells using low levels of FBSP, extensive trace elements or no serum protein.

MCDB 302 MEDIUM, Product No. M2021 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
Ammonium Metavanadate	0.00000117
Calcium Chloride•2H_2O	0.08821
Cupric Sulfate•5H_2O	0.0000025
Ferrous Sulfate•7H_2O	0.000834
Magnesium Chloride (anhydrous)	0.05713
Manganese Sulfate	0.000000151
Molybdic Acid•4H_2O (ammonium)	0.0000124
Potassium Chloride	0.22365
Sodium Chloride	7.599
Sodium Phosphate Dibasic (anhydrous)	0.14198
Sodium Selenite	0.00000173
Zinc Sulfate•7H_2O	0.000863
L-Alanine	0.00891
L-Arginine•HCl	0.2107
L-Asparagine•H_2O	0.015
L-Aspartic Acid	0.01331
L-Cysteine•HCl•H_2O	0.01756
L-Glutamic Acid	0.01471
L-Glutamine	0.4386
Glycine	0.00751
L-Histidine•HCl•H_2O	0.02096
L-Isoleucine	0.00394
L-Leucine	0.01312
L-Lysine•HCl	0.03654
L-Methionine	0.00448
L-Phenylalanine	0.00496
L-Proline	0.03453
L-Serine	0.01051
L-Threonine	0.01191
L-Tryptophan	0.00204
L-Tyrosine•2Na	0.007896
L-Valine	0.01172
D-Biotin	0.00000733
Choline Chloride	0.01396
Folic Acid	0.001324
myo-Inositol	0.01802
Niacinamide	0.0000366
D-Pantothenic Acid (hemicalcium)	0.000238
Pyridoxine•HCl	0.0000617
Riboflavin	0.0000376
Thiamine•HCl	0.000337
Vitamin B-12	0.00136
D-Glucose	1.8016
Hypoxanthine	0.004083
Linoleic Acid	0.0000841
Phenol Red•Na	0.001242
Putrescine•2HCl	0.000161
Pyruvic Acid•Na	0.1101
Thioctic Acid	0.000206

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 15-20EC.
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 1.18 g sodium bicarbonate or 15.7 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 2-8EC under dry conditions and liquid medium at 2-8EC 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 room temperature [without sodium bicarbonate]	6.7 ± 0.3
pH at room temperature [with sodium bicarbonate]	7.6 ± 0.3
Osmolality [without sodium bicarbonate]	268 mOsm/kg H_2O ± 5%
Osmolality [with sodium bicarbonate]	290 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. Boyce, S.T. and Ham, R.G., (1983). Calcium-Regulated Differentiation of Normal Human Epidermal Keratinocytes in Chemically Defined Clonal Culture and Serum-Free Serial Culture. J. Invest. Dermatol, 81, 33-40.
2. McKeehan, W.L. and Ham, R.G., (1976). Stimulation of Clonal Growth of Normal Fibrblasts with Substrata Coated with Basic Polymers. J. Cell Biol., 71, 727-734.
3. Hamilton, W.G. and Ham, R.G., (1977). Clonal Growth of Chinese Hamster Ovary Cell Lines in Protein-Free Media. In Vitro, 13:9, 537-547.

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