ProductInformation

CMRL-1066
With L-Glutamine, Without Sodium Bicarbonate

Product Number **C0422**
Storage Temperature 2-8°C

Product Description
CMRL-1066 is a chemically defined medium developed in the late 1950's by Connaught Medical Research Laboratories. A less complex and extensively modified version of Medium 199, CMRL-1066 was designed initially for use with L-strain cells in unsupplemented culture. It has been used to maintain monolayer growth of permanent cell lines for years without protein supplementation. Although developed for use in serum free cell culture, CMRL-1066 can be supplemented with serum and used to support the growth of many types of cells.

CMRL-1066, Product No. C0422 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
L-Alanine	0.025
L-Arginine	0.05787
L-Aspartic Acid	0.03
L-Cysteine•HCl• H_2O	0.26
L-Cystine	0.02
L-Glutamic Acid	0.075
L-Glutamine	0.1
Glycine	0.05
L-Histidine HCl• H_2O	0.02
Trans-4-Hydroxy-L-Proline	0.01
L-Isoleucine	0.02
L-Leucine	0.06
L-Lysine•HCl	0.07
L-Methionine	0.015
L-Phenylalanine	0.025
L-Proline	0.04
L-Serine	0.025
L-Threonine	0.03
L-Tryptophan	0.01
L-Tyrosine	0.04
L-Valine	0.025
L-Ascorbic Acid	0.05
PABA	0.00005
D-Biotin	0.00001
Choline Chloride	0.0005
Coenzyme A•Na	0.0025
Cocarboxylase	0.001
2'-Deoxyadenosine	0.01
2'-Deoxyguanosine	0.01
2'-Deoxycytidine•HCl	0.0116
Flavin Aadenine Dinucleotide•2Na	0.000106
Folic Acid	0.00001
myo-Inositol	0.00005
5-Methyldeoxycytidine	0.0001
β-NAD	0.007
β-NADP•Na	0.001
Niacinamide	0.000025
Nicotinic Acid	0.000025
D-Pantothenic Acid [hemicalcium]	0.00001
Pyridoxal•HCl	0.000025
Pyridoxine•HCl	0.000025
Riboflavin	0.00001
Thiamine•HCl	0.00001
Thymidine	0.01
Uridine-5-Triphosphate•Na	0.001
Calcium Chloride [Anhydrous]	0.2
Magnesium Sulfate [Anhydrous]	0.09769
Potassium Chloride	0.4
Sodium Acetate [Anhydrous]	0.05
Sodium Chloride	6.8
Sodium Phosphate Monobasic [Anhydrous]	0.122
D-Glucose	1.0
Phenol Red•Na	0.02124
Glutathione	0.01
D-Glucuronic Acid•Na	0.00388
Cholesterol	0.0002
Tween 80	0.005

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 2.2 g sodium bicarbonate or 29.3 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]	3.3 ± 0.3
pH at RT [with sodium bicarbonate]	7.0 ± 0.3
Osmolality [without sodium bicarbonate]	241 mOsm/kg H_2O ± 5%
Osmolality [with sodium bicarbonate]	284 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. Parker R. C. et al. Altered Cell Strains In Continuous Culture: A General Survey In: Special Publications of the New York Academy of Sciences. Publisher: N.Y. Acad. of Sci. Ed.: Whitelock O. Vol 5, 303-313, 1957.
2. Healy, G. M., and Parker, R.C., An Improved Chemically Defined Basal Medium (CMRL-1415) For Newly Explanted Mouse Embryo Cells Journal of Cell Biology 30: 531-538, 1966.
3. Methods of Tissue Culture, Third Edition. Harper and Row Publishers Inc. New York Ed. R. C. Parker, 62-80.

C0422 10/98