ProductInformation

MCCOY'S 5A MEDIUM
With L-Glutamine, Without Phenol Red and Sodium Bicarbonate

Product Number **M9270**
Storage Temperature 2-8°C

Product Description
In 1959, McCoy and his coworkers reported the amino acid requirements for in vitro cultivation of Novikoff Hepatoma Cells. These studies were performed using Basal Medium 5A, formulated to support Walker Carcinosarcoma 256 Cells, and subsequently modified to create a new medium known as McCoy's 5A Medium. Hsu and Kellogg employed this medium to support growth of primary cultures derived from normal bone marrow, skin, gingiva, testes, mouse kidney, omentum, adrenal glands, lung, spleen, rat embryos, and other tissues.

MCCOY'S 5A MEDIUM, Product No. M-9270 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	0.1324324
Magnesium Sulfate (anhydrous)	0.0976876
Potassium Chloride	0.4
Sodium Chloride	6.46
Sodium Phosphate Monobasic (anhydrous)	0.504
L-Alanine	0.01336
L-Arginine•HCl	0.04214
L-Asparagine•H_2O	0.04503
L-Aspartic Acid	0.01997
L-Cysteine	0.02424
L-Glutamic Acid	0.02207
L-Glutamine	0.21915
Glycine	0.00751
L-Histidine•HCl• H_2O	0.02096
Trans-4-Hydroxy-L-proline	0.01967
L-Isoleucine	0.03936
L-Leucine	0.03936
L-Lysine•HCl	0.03654
L-Methionine	0.01492
L-Phenylalanine	0.01652
L-Proline	0.01727
L-Serine	0.02628
L-Threonine	0.01787
L-Tryptophan	0.00306
L-Tyrosine•2Na•2 H_2O	0.0261
L-Valine	0.01757
Ascorbic Acid	0.0005625
p-Aminobenzoic Acid	0.001
D-Biotin	0.0002
Choline Chloride	0.005
Folic Acid	0.01
myo-Inositol	0.036
Niacinamide	0.0005
Nicotinic Acid	0.0005
D-Pantothenic Acid (hemicalcium)	0.0002
Pyridoxal•HCl	0.0005
Pyridoxine•HCl	0.0005
Riboflavin	0.0002
Thiamine•HCl	0.0002
Vitamin B-12	0.002
Peptone	0.6
D-Glucose	3.0
Glutathione (reduced)	0.0005

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 [W-3500]
Sodium Bicarbonate [S-5761] or
Sodium Bicarbonate Solution, 7.5% [S-8761]
1N Hydrochloric Acid [H-9892]
1N Sodium Hydroxide [S-2770]
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]	5.1 ± 0.3
pH at room temperature [with sodium bicarbonate]	7.1 ± 0.3
Osmolality [without sodium bicarbonate]	254 mOsm/kg H2O ± 5%
Osmolality [with sodium bicarbonate]	290 mOsm/kg H2O ± 5%
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. McCoy, T.A., Maxwell, M. and Kruse, P.F., (1959). Amino Acid Requirement of the Novioff Hepatoma In Vitro. 100, 115-118.
2. Patterson, M.K. and Dell'orco, R.T., (1978). Preparation of McCoy's Medium 5A. Tissue Culture Association Manual. 4, 737-740.
3. Hsu, T.C. and Kellogg, D.S., (1960). Primary Cultivation and Continuous Propagation In Vitro of Tissues from Small Biopsy Specimens. J.N.C.I. 25, 221-231.
4. Iwakata, S. and Grace, J.T., (1964). Cultivation in Vitro of Myeoblasts from Human Leukemia. New York State Journal of Med.18, 2279-2282.
5. Morton, H..J., (1970).A Survey of Commercially Available Tissue Culture Media.In Vitro. 6, 89.