ProductInformation

MINIMUM ESSENTIAL MEDIUM EAGLE
D-VALINE MODIFICATION

Product Number **M7395**
Storage Temperature 2-8°C

Product Description
Minimum Essential Medium (MEM), developed by Harry Eagle, is one of the most widely used of all synthetic cell culture media. Early attempts to cultivate normal mammalian fibroblasts and certain subtypes of HeLa cells revealed that they had specific nutritional requirements that could not be met by Eagle's Basal Medium (BME). Subsequent studies using these and other cells in culture indicated that additions to BME could be made to aid growth of a wider variety of fastidious cells. MEM, which incorporates these modifications, includes higher concentrations of amino acids. MEM has been used for cultivation of a wide variety of cells grown in monolayers. Optional supplementation of non-essential amino acids to the formulations that incorporate either Hanks' or Earle's salts has broadened the usefulness of this medium. The formulation has been further modified by optional elimination of calcium to permit growth of cells in suspension culture.

MINIMUM ESSENTIAL MEDIUM EAGLE, Product No. M 7395 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•2H_2O	0.265
Magnesium Sulfate (anhydrous)	0.09767
Potassium Chloride	0.4
Sodium Chloride	6.8
Sodium Phosphate Monobasic (anhydrous)	0.122
L-Arginine•HCl	0.126
L-Cystine•2HCl	0.0313
L-Glutamine	0.292
L-Histidine•HCl•H_2O	0.042
L-Isoleucine	0.052
L-Leucine	0.052
L-Lysine•HCl	0.0725
L-Methionine	0.015
L-Phenylalanine	0.032
L-Threonine	0.048
L-Tryptophan	0.01
L-Tyrosine•2Na•2H_2O	0.0519
D-Valine	0.092
Choline Chloride	0.001
Folic Acid	0.001
myo-Inositol	0.002
Niacinamide	0.001
D-Pantothenic Acid (hemicalcium)	0.001
Pyridoxal•HCl	0.001
Riboflavin	0.0001
Thiamine•HCl	0.001
Glucose	1.0
Phenol Red•Na	0.011

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

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]	5.8 ± 0.3
pH at room temperature [with sodium bicarbonate]	7.5 ± 0.3
Osmolality [without sodium bicarbonate]	250 mOsm/kg H_2O ± 5%
Osmolality [with sodium bicarbonate]	290 mOsm/kg H_2O ± 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. Eagle, H. et al (1956) myo-Inositol as an Essential Growth Factor for Normal and Malignant Human Cells in Tissue Culture. J.Biol. Chem. 214, 845-847.
2. Eagle, H.(1976) Media for Animal Cell Culture. Tissue Culture Association Manual. 3, 517-520.
3. Eagle, H. (1959). Amino Acid Metabolism in Mammalian Cell Cultures. Science. 130, 432-437.
4. Eagle, H. (1955) Nutrition Needs of Mammalian Cells in Culture. Science. 122, 501.
5. Gilbert, S.F. and Migeon, B.R. (1975) D-valine as a selective agent for normal human and rodent epithelial cells in culture. Cell. 5, 11-17.

7H027