



Minimum Essential Medium Eagle (MEM) (Alpha modification)

With L-Glutamine

Without Deoxyribonucleosides, Ribonucleosides, Phenol red and Sodium bicarbonate Product Code: AT300

Product Description:

Minimum Essential Medium Eagle (MEM) is a modification of Basal Medium Eagle (BME). It was developed by Harry Eagle to meet the specific nutritional requirements of certain subtypes of HeLa cells and normal mammalian fibroblasts. MEM includes higher concentration of amino acids so as to closely approximate the protein composition of cultured mammalian cells. MEM can be used either with Earle's salts or Hanks' salts and can also be supplemented with non-essential amino acids (NEAA). This medium can be further modified by eliminating calcium to facilitate growth of cells in suspension cultures.

AT300 is alpha modification of Minimum Essential Medium Eagle with l-glutamine. It does not contain deoxyribonucleosides, ribonucleosides and phenol red and sodium bicarbonate. Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

Composition:

Ingredients	mg/L
INORGANIC SALTS	_
Calcium chloride dihydrate	265.000
Magnesium sulphate anhydrous	97.720
Potassium chloride	400.000
Sodium chloride	6800.000
Sodium dihydrogen phosphate anhydrous	122.000
AMINO ACIDS	
Glycine	50.000
L-Alanine	25.000
L-Arginine hydrochloride	126.000
L-Asparagine monohydrate	50.000
L-Aspartic acid	30.000
L-Cysteine hydrochloride monohydrate	100.000
L-Cystine dihydrochloride	31.300
L-Glutamic acid	75.000
L-Glutamine	292.000
L-Histidine hydrochloride monohydrate	42.000

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L-Isoleucine	52.000
L-Leucine	72.500
L-Lysine hydrochloride	15.000
L-Methionine	32.000
L-Phenylalanine	40.000
L-Proline	25.000
L-Serine	48.000
L-Threonine	10.000
L-Tryptophan	51.900
L-Tyrosine disodium salt dihydrate	46.000
L-Valine	
VITAMINS	
Choline chloride	1.000
D-Biotin	0.100
D-Ca-Pantothenate	1.000
Folic acid	1.000
L-Ascorbic acid	50.000
Nicotinamide	1.000
Pyridoxal hydrochloride	1.000
Riboflavin	0.100
Thiamine hydrochloride	1.000
Vitamin B12	1.360
i-Inositol	2.000
OTHERS	
D-Glucose	1000.000
Lipoic acid	0.200
Sodium pyruvate	110.000

Directions:

- 1. Suspend 10.1gms in 900 ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
- 2. Add 2.2gms of sodium bicarbonate powder (TC230) or 29.3ml of 7.5% sodium bicarbonate solution (TCL013) for 1 litre of medium and stir until dissolved.
- 3. Adjust the pH to 0.2 0.3 pH units below the desired pH using 1N HCl or 1N NaOH since the pH tends to rise during filtration.
- 4. Make up the final volume to 1000ml with tissue culture grade water.

- 5. Sterilize the medium immediately by filtering through a sterile membrane filter with a porosity of 0.22 micron or less, using positive pressure rather than vacuum to minimize the loss of carbon dioxide.
- 6. Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into sterile containers.
- 7. Store liquid medium at 2-8°C and in dark till use.

Material required but not provided:

Tissue culture grade water (TCL010) Sodium bicarbonate (TC230) Sodium bicarbonate solution, 7.5% (TCL013) 1N Hydrochloric acid (TCL003) 1N Sodium hydroxide (TCL002) Foetal bovine serum (RM1112/RM10432)

Quality Control:

Appearance

White to off-white, homogenous powder

Solubility

Clear solution at 10.1 gms/L.

pH without Sodium Bicarbonate 3.50 -4.10

pH with Sodium Bicarbonate

7.00 - 7.60

Osmolality without Sodium Bicarbonate (mOsm Kg/H₂O) 250.00 -290.00

Osmolality with Sodium Bicarbonate (mOsm Kg/H_2O) 300.00 - 340.00

Cultural Response

The growth promotion capacity of the medium is assessed qualitatively by analyzing the cells for the morphology and quantitatively by estimating the cell counts.

Endotoxin Content

NMT 1EU/ml

Storage and Shelf Life:

1. All the powdered media and prepared liquid culture media should be stored at 2-8°C. Use before the expiry date. In spite of above recommended storage condition, certain powdered medium may show some signs of deterioration /degradation in certain instances. This can be indicated by change in colour, change in appearance and presence of particulate matter and haziness after dissolution.

- 2. Preparation of concentrated medium is not recommended since free base amino acids and salt complexes having low solubility may precipitate in concentrated medium.
- 3. pH and sodium bicarbonate concentration of the prepared medium are critical factors affecting cell growth. This is also influenced by amount of medium and volume of culture vessel used (surface to volume ratio). For example, in large bottles, such as Roux bottles pH tends to rise perceptibly as significant volume of carbon dioxide is released. Therefore, optimal conditions of pH, sodium bicarbonate concentration, surface to volume ratio must be determined for each cell type. We recommend stringent monitoring of pH. If needed, pH can be adjusted by using sterile 1N HCl or 1N NaOH or by bubbling in carbon dioxide.
- 4. If required, supplements can be added to the medium prior to or after filter sterilization observing sterility precautions. Shelf life of the medium will depend on the nature of supplement added to the medium.

Disclaimer: Revision: 03 / 2022

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