

### Technical Information

#### Minimum Essential Medium Eagle (MEM) (Modified for autoclaving) With Earle's salts and NEAA Without L-Glutamine and Sodium bicarbonate

**Product Code : AT1017A**

**Application:** - Minimum Essential Medium (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 Hank's salts and can also be additionally supplemented with non-essential amino acids (NEAA). This medium can be further modified by eliminating calcium to facilitate growth of cells in suspension cultures. AT1017A is Minimum Essential Medium Eagle with Earle's balanced salts, NEAA and phenol red and is modified for autoclaving. Autoclavable media offer a convenient alternative to membrane sterilized liquid medium. It is modified to include heat stable components to ensure that product efficacy is maintained after autoclaving. L-glutamine is heat labile, hence has been omitted from the formulation. Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

### Composition\*\*

Ingredients	Gms / Litre
<b>INORGANIC SALTS</b>	
Calcium chloride dihydrate	265.000
Magnesium sulphate anhydrous	97.720
Potassium chloride	400.000
Sodium chloride	6800.000
Sodium phosphate, monobasic	122.000
<b>AMINO ACIDS</b>	
Glycine	7.500
L-Alanine	8.900
L-Arginine hydrochloride	126.000
L-Asparagine monohydrate	15.000
L-Aspartic acid	13.300
L-Cystine dihydrochloride	31.300
L-Glutamic Acid	14.700
L-Histidine hydrochloride monohydrate	42.000
L-Isoleucine	52.000
L-Leucine	52.000
L-Lysine hydrochloride	72.500
L-Methionine	15.000
L-Phenylalanine	32.000
L-Proline	11.500
L-Serine	10.500
L-Threonine	48.000
L-Tryptophan	10.000
L-Tyrosine disodium salt	52.000
L-Valine	46.000

### VITAMINS

Choline bitartrate	1.800
D-Ca-Pantothenate	1.000
Folic acid	1.000
Nicotinamide	1.000
Pyridoxal hydrochloride	1.000
Riboflavin	0.100
Thiamine hydrochloride	1.000
i-Inositol	2.000

### OTHERS

D-Glucose	1000.000
Phenol red sodium salt	6.300
Sodium succinate	100.000
Succinic acid	75.000

## Methodology

1. Suspend 9.5gms of the powder in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved.
2. Adjust the pH to 4.0 before autoclaving.
3. Make up the volume with tissue culture grade water. Subtract the volumes of 7.5% sodium bicarbonate solution and 200mM L-glutamine solution from the final volume.
4. Autoclave medium at 121°C at 15psi for 15minutes.
5. Remove the medium promptly from the autoclave to avoid extended heating or evaporation.
6. Allow to cool at room temperature.
7. Add 29.3ml of 7.5% sodium bicarbonate solution (TCL1013) and 10ml of 200mM L-glutamine solution (TCL1012) to the final volume of the medium being prepared.
8. If necessary, adjust the pH using sterile 1N NaOH (TCL1002) or 1N HCl (TCL1003).
9. Store liquid medium at 2-8°C and in dark till use.

## Material required but not provided

Tissue culture grade water (TCL1010)  
Sodium bicarbonate solution, 7.5% (TCL1013)  
1N Hydrochloric acid (TCL1003)  
1N Sodium hydroxide (TCL1002)  
200mM L-Glutamine solution (TCL1012)  
Foetal bovine serum (BA3112/BA30432)

## Quality Control

### Appearance

Off-white to Creamish white, homogenous powder.

### Solubility

Clear solution at 9.5gms/L

### pH without Sodium Bicarbonate

4.10-4.70

### pH with Sodium Bicarbonate

7.30-7.90

### Osmolality without Sodium Bicarbonate

240.00 -280.00

### Osmolality with Sodium Bicarbonate

285.00-325.00

### 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 and comparing it with a control medium through minimum three subcultures.

### Endotoxin Content

NMT 5EU/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 :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conforms solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate.
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