

### Technical Information

#### Minimum Essential Medium Eagle (MEM) With Earle's salts, NEAA, L-Glutamine and 25mM HEPES Buffer Without Sodium bicarbonate

#### Product Code: AT1153

**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 Hanks 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.

AT1153 is Minimum Essential Medium Eagle with Earle's balanced salts, L-glutamine, non-essential amino acids and 25mM HEPES buffer. HEPES, a zwitterionic buffer having a pKa of 7.3 at 37°C prevents the initial rise in pH that tends to occur at the initiation of a culture and increases the buffering capacity of the medium. Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

#### Composition\*\*

Ingredients	mg/Litre
<b>INORGANIC SALTS</b>	
Calcium chloride dihydrate	265.000
Magnesium sulphate anhydrous	97.720
Potassium chloride	400.000
Sodium chloride	6800.000
Sodium dihydrogen phosphate anhydrous	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-Cysteine dihydrochloride	31.300
L-Glutamic acid	14.700
L-Glutamine	292.000
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-Prolin	11.500
L-Serine	10.500
L-Threonine	48.000
L-Tryptophan	10.000
L-Tyrosine disodium salt	51.900

L-Valine	46.000
<b>VITAMINS</b>	
Choline chloride	1.000
D-Ca-Pantothenate	1.000
Folic acid	1.000
Nicotinamide	1.000
Pyridoxal	1.000
Riboflavin	0.100
Thiamine hydrochloride	1.000
i-Inositol	2.000
<b>OTHERS</b>	
D-Glucose	1000.000
HEPES Buffer	5958.000
Phenol red sodium salt	11.000
Sodium pyruvate	110.000

### Methodology

1. Suspend 15.6gms in 900ml 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 (TC1230) or 29.3ml of 7.5% Sodium bicarbonate solution (TCL1013) 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 (TCL1010)  
 Sodium bicarbonate powder (TC1230)  
 Sodium bicarbonate solution, 7.5% (TCL1013)  
 1N Hydrochloric acid (TCL1003)  
 1N Sodium hydroxide (TCL1002)  
 Foetal bovine serum (BA3112/BA12432)

### Quality control

#### Appearance

Off-white to Creamish white, homogenous powder.

#### Solubility

Clear solution at 15.6gms/L.

#### pH without Sodium Bicarbonate

5.20-5.80

#### pH with Sodium Bicarbonate

6.70-7.30

### Osmolality without Sodium Bicarbonate

270.00 -310.00

### Osmolality with Sodium Bicarbonate

310.00 -350.00

### 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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- Do not use the products if it fails to meet specifications for identity and performance parameters.