

Minimum Essential Medium Eagle (MEM)

(Modified for Autoclaving)

With Hank's salts and NEAA

Without L-Glutamine and Sodium bicarbonate

Product Code: AT045A

Product Description :

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.

AT045A is Minimum Essential Medium Eagle with Hank'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 | mg/L |
|---------------------------------------|----------|
| INORGANIC SALTS | |
| Calcium chloride dihydrate | 185.000 |
| Disodium hydrogen phosphate anhydrous | 47.880 |
| Magnesium sulphate anhydrous | 97.720 |
| Potassium chloride | 400.000 |
| Potassium dihydrogen phosphate | 60.000 |
| Sodium chloride | 8000.000 |
| AMINO ACIDS | |
| 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 |

Directions :

1. Suspend 10.6gms 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. Adjust the pH to 7.2 with sterile 1N NaOH (TCL002) observing all sterile precautions.
8. Add 4.7ml of 7.5% sodium bicarbonate solution (TCL013) and 10ml of 200mM L-glutamine solution (TCL012) to the final volume of the medium being prepared.
9. If necessary, adjust the pH using sterile 1N NaOH (TCL002) or 1N HCl (TCL003).
10. Store liquid medium at 2-8°C and in dark till use.

Material required but not provided :

Tissue culture grade water (TCL010)
Sodium bicarbonate solution, 7.5% (TCL013)
1N Hydrochloric acid (TCL003)
1N Sodium hydroxide (TCL002)
200mM L-Glutamine solution (TCL012)
Fetal bovine serum (RM1112/RM10432)

Quality Control:

Appearance

Off-white to Creamish white, homogenous powder.

Solubility

Clear solution at 10.6 gms/L.

pH without Sodium Bicarbonate

4.60 -5.20

pH with Sodium Bicarbonate

6.30 -6.90

Osmolality without Sodium Bicarbonate

270.00 -310.00

Osmolality with Sodium Bicarbonate

290.00 -330.00

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.

Revision : 1 / 2012



Disclaimer :

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