

Technical Information

Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12 Ham (DMEM / F12, 1:1 mixture)
w/ L-Glutamine, 15mM HEPES buffer and Trace elements w/o Sodium bicarbonate

Product Code : AT1139

Application:-

Dulbecco's Modified Eagle Medium / Nutrient Mixture F-12 Ham (DMEM/F12, 1:1 mixture) was originally formulated for rat neuroblastoma cells and MDCK cells. The mixture is extremely nutritious and supports growth of a wide variety of cells including certain epithelial, endothelial and granulosa cells.

AT1139 is DMEM/ Nutrient Mixture F-12 Ham with L-glutamine, 15mM HEPES buffer and trace elements. 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

INORGANIC SALTS

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|---|----------|
| Ammonium metavanadate | 0.00058 |
| Ammonium molybdate tetrahydrate | 0.00618 |
| Calcium chloride dihydrate | 154.500 |
| Copper sulphate pentahydrate | 0.0013 |
| Disodium hydrogen phosphate | 71.020 |
| Ferric nitrate nonahydrate | 0.050 |
| Ferrous sulphate heptahydrate | 0.417 |
| Magnesium chloride hexahydrate | 61.200 |
| Magnesium sulphate anhydrous | 48.840 |
| Manganese sulphate | 0.000151 |
| Nickel chloride | 0.00012 |
| Potassium chloride | 311.800 |
| Sodium chloride | 6996.000 |
| Sodium dihydrogen phosphate monohydrate | 54.300 |
| Sodium metasilicate nonahydrate | 0.0142 |
| Sodium selenite | 0.00519 |
| Stannous chloride dihydrate | 0.00011 |
| Zinc sulphate heptahydrate | 0.432 |

AMINO ACIDS

| | |
|----------------------------|---------|
| Glycine | 18.750 |
| L-Alanine | 4.450 |
| L-Arginine hydrochloride | 147.500 |
| L-Asparagine monohydrate | 7.500 |
| L-Aspartic acid | 6.650 |
| L-Cysteine dihydrochloride | 17.560 |

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|---------------------------------------|----------|
| L-Cystine hydrochloride monohydrate | 31.290 |
| L-Glutamic acid 7.350 L-Glutamine | 365.000 |
| L-Histidine hydrochloride monohydrate | 31.480 |
| L-Isoleucine | 54.470 |
| L-Leucine | 59.050 |
| L-Lysine hydrochloride | 91.250 |
| L-Methionine | 17.240 |
| L-Phenylalanine | 35.480 |
| L-Proline | 17.250 |
| L-Serine | 26.250 |
| L-Threonine | 53.450 |
| L-Tryptophan | 9.020 |
| L-Tyrosine disodium salt | 48.100 |
| L-Valine | 52.850 |
| VITAMINS | |
| Ca-D-Pantothenic acid | 2.240 |
| Choline chloride | 8.980 |
| D-Biotin | 0.0035 |
| Folic acid | 2.660 |
| Niacinamide | 2.020 |
| Pyridoxal hydrochloride | 2.000 |
| Pyridoxine hydrochloride | 0.031 |
| Riboflavin | 0.219 |
| Thiamine hydrochloride | 2.170 |
| Vitamin B12 | 0.680 |
| myo-Inositol | 12.600 |
| OTHERS | |
| D-Glucose | 3151.000 |
| DL-Thioctic acid | 0.105 |
| HEPES buffer | 3574.500 |
| Hypoxanthine | 2.400 |
| Linoleic acid | 0.042 |
| Phenol red sodium salt | 8.630 |
| Putrescine hydrochloride | 0.081 |
| Sodium pyruvate | 110.000 |
| Thymidine | 0.365 |

Methodology

1. Suspend 15.7gms in 900 ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 1.2gms of sodium bicarbonate powder (TC1230) or 16.0ml of 7.5% sodium bicarbonate solution (TCL1013) for 1litre 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 (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 15.7 gms/L.

pH without Sodium Bicarbonate

5.50 -6.10

pH with Sodium Bicarbonate

6.60 -7.20

Osmolality without Sodium Bicarbonate

270.00 -310.00

Osmolality with Sodium Bicarbonate

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 and comparing it with a control medium through minimum three subcultures

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.
- **Central Drug House Pvt. Ltd.** reserves the right to make changes to specifications and information related to the products at any time.
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- Do not use the products if it fails to meet specifications for identity and performance parameters.