



Dulbecco's Modified Eagle Medium/ Nutrient Mixture F12 Ham (DMEM/F12, 1:1 mixture)

With L-Glutamine

Without HEPES buffer, Trace elements and Sodium bicarbonate

Product Code: AT155

Product Description:

Dulbecco's Modified Eagle Medium / Nutrient Mixture F12 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.

AT155 is DMEM/ Nutrient Mixture F-12 Ham with L-glutamine. It does not contain HEPES buffer, phenol red and trace elements. 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 | 154.500 |
| Copper sulphate pentahydrate | 0.0013 |
| Disodium hydrogen phosphate, anhydrous | 71.020 |
| Ferric nitrate nonahydrate | 0.050 |
| Ferrous sulphate heptahydrate | 0.417 |
| Magnesium chloride anhydrous | 61.200 |
| Magnesium sulphate anhydrous | 48.840 |
| Potassium chloride | 311.800 |
| Sodium chloride | 6996.000 |
| Sodium dihydrogen phosphate anhydrous | 54.300 |
| 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 |
| 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 | |
| Choline chloride | 8.980 |
| D-Biotin | 0.0035 |
| D-Pantothenic acid (hemicalcium) | 2.240 |
| 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 |
| Hypoxanthine | 2.400 |
| Linoleic acid | 0.042 |
| Phenol red Sodium Salt | 8.630 |
| Putrescine hydrochloride | 0.081 |
| Sodium pyruvate | 110.000 |
| Thymidine | 0.365 |
| | |

Directions:

- 1. Suspend 12.1gms in 900ml 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 (TC230) or 16ml 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

Off-white to Creamish white, homogenous powder.

Solubility

Clear solution at 12.1 gms/L.

pH without Sodium Bicarbonate 6.00 - 6.60

pH with Sodium Bicarbonate 7.20 - 7.80

Osmolality without Sodium Bicarbonate 260.00 - 300.00

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. Inspite 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 filter sterilization observing sterility precautions. Shelf life of the medium will depend on the nature of supplement added to the medium.

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