

Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12 Ham (DMEM/ F12, 3:1 Mixture)

With L-Glutamine

Without Calcium Chloride, HEPES buffer and Sodium bicarbonate

Product Code: AT189

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.

AT189 is Dulbecco's Modified Eagle Medium / Nutrient Mixture F-12 Ham with L-glutamine. It does not contain calcium chloride, HEPES buffer and sodium bicarbonate. 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	
Copper sulphate pentahydrate	0.000625
Disodium hydrogen phosphate anhydrous	35.510
Ferric nitrate nonahydrate	0.075
Ferrous sulphate heptahydrate	0.209
Magnesium chloride anhydrous	14.412
Magnesium sulphate anhydrous	73.290
Potassium chloride	356.000
Sodium chloride	6699.750
Sodium dihydrogen phosphate anhydrous	81.750
Zinc sulphate heptahydrate	0.215
AMINO ACIDS	
Glycine	24.380
L-Alanine	2.227
L-Arginine hydrochloride	115.520
L-Asparagine anhydrous	3.752
L-Aspartic acid	3.325
L-Cysteine hydrochloride monohydrate	8.780
L-Cystine dihydrochloride	46.927
L-Glutamic acid	3.675
L-Glutamine	474.500
L-Histidine hydrochloride monohydrate	36.740

L-Isoleucine	79.735
L-Leucine	82.025
L-Lysine hydrochloride	118.625
L-Methionine	23.625
L-Phenylalanine	50.740
L-Proline	8.625
L-Serine	34.125
L-Threonine	74.225
L-Tryptophan	12.510
L-Tyrosine disodium salt	79.795
L-Valine	73.425

VITAMINS

Choline chloride	6.490
D-Biotin	0.001825
D-Ca-Pantothenate	3.120
Folic acid	3.330
Niacinamide	3.000
Pyridoxine hydrochloride	3.015
Riboflavin	0.309
Thiamine hydrochloride	3.085
Vitamin B12	0.340
myo-Inositol	9.900

OTHERS

D-Glucose	3825.500
DL-Thioctic acid	0.053
Hypoxanthine	1.200
Linoleic acid	0.021
Phenol red sodium salt	12.235
Putrescine dihydrochloride	0.040
Sodium pyruvate	27.525
Thymidine	0.183

Directions :

1. Suspend 12.5gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 3.07gms of sodium bicarbonate powder (TC230) or 40.8ml 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 12.5gms/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

320.00 - 360.00

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.

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