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Product Specification

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Technical Information

Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 Ham (DMEM/F12, 1:1 mixture) With L-Glutamine, 15mM HEPES buffer and Trace elements Without Phenol red and Sodium bicarbonate

Product Code: AT1192

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.

AT1192 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 37ºC prevents the initial rise in pH that tends to occur at the initiation of a culture and increases the buffering capacity of the medium. It does not contain phenol red 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/Litre
INORGANIC SALTS	
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 metasillicate nonahydrate	0.0142
Sodium selenite	0.00519
Stannous chloride dihydrate	0.00011
Zinc sulphate heptahydrate	0.432

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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 dihydrate	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
Putrescine hydrochloride	0.081
Sodium pyruvate	110.000
Thymidine	0.365

Methodology

1. Suspend 15.7 gms 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 (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 15.7gms/L.

pH without Sodium Bicarbonate

5.50 -6.10

pH with Sodium Bicarbonate

6.60 -7.20

Osmolality without Sodium Bicarbonate

280.00 -320.00

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.

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 spiteof above recommended storage condition, certain powdered medium may show some signs of deterioration /degradationin 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 preparedmedium are critical factors affecting cell growth. This is also influenced by amount of medium and volume of culturevessel 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 priorto 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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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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