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

Without Calcium chloride, HEPES buffer, L-Glutamine and Sodium bicarbonate

Product Code: AT189A

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.

AT189A is Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12 Ham 3:1 mixture. It does not contain L-glutamine, 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:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper sulphate pentahydrate</td>
<td>0.00625</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate anhydrous</td>
<td>35.510</td>
</tr>
<tr>
<td>Ferric nitrate nonahydrate</td>
<td>0.075</td>
</tr>
<tr>
<td>Ferrous sulphate heptahydrate Magnesium chloride anhydrous</td>
<td>0.209</td>
</tr>
<tr>
<td>Magnesium sulphate anhydrous</td>
<td>14.412</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>73.290</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>356.000</td>
</tr>
<tr>
<td>Sodium dihydrogen phosphate anhydrous</td>
<td>6699.750</td>
</tr>
<tr>
<td>Zinc sulphate heptahydrate</td>
<td>81.750</td>
</tr>
<tr>
<td>AMINO ACIDS</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>24.380</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>2.227</td>
</tr>
<tr>
<td>L-Arginine hydrochloride</td>
<td>115.520</td>
</tr>
<tr>
<td>L-Asparagine anhydrous</td>
<td>3.752</td>
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<tr>
<td>L-Aspartic acid</td>
<td>3.325</td>
</tr>
<tr>
<td>L-Cysteine hydrochloride monohydrate</td>
<td>8.780</td>
</tr>
<tr>
<td>L-Cystine dihydrochloride</td>
<td>46.927</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>3.675</td>
</tr>
<tr>
<td>L-Histidine hydrochloride monohydrate</td>
<td>36.740</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>79.735</td>
</tr>
</tbody>
</table>

VITAMINS
- Choline chloride: 6.490 mg/L
- Biotin: 0.001825 mg/L
- D-Ca-Pantothenate: 3.120 mg/L
- Folic acid: 3.330 mg/L
- Nicotinamide: 3.000 mg/L
- Pyridoxine hydrochloride: 3.015 mg/L
- Riboflavin: 0.309 mg/L
- Thiamine hydrochloride: 3.085 mg/L
- Vitamin B12: 0.340 mg/L
- myo-Inositol: 9.900 mg/L

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

Directions:
1. Suspend 12.0gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 0.4745gms of L-Glutamine powder (TC243) or 16.25ml of 200mM L-Glutamine solution (TCL012) and 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)
L-Glutamine (TC243)
L-Glutamine Solution, 200mM (TCL012)
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
White to light pink, homogenous powder.

Solubility
Clear solution at 12.0gms/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.

Endotoxin Content
NMT 1 EU/ml

Revision : 0 / 2017

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