Nutrient Mixture F-12 Ham
Without L-Glutamine and Sodium bicarbonate

Product Code: AT025A

Product Description:
Ham's Nutrient Mixtures were originally developed for single cell plating of near diploid Chinese hamster ovary (CHO) cells and mouse L-cells. Both F-10 and F-12 are formulated for use with or without serum, depending on the type of cells being cultured.

Ham's Nutrient Mixtures F12 was originally designed for serial propagation and cloning of two CHO cell lines namely, CHD-3 and CHL-1 and mouse L cells. It is the medium of choice for the growth of cells of rodent origin and for cloning of myeloma and hybridoma cells. This medium is also the medium of choice for clonal toxicity assay using CHO cells.

AT025A is Nutrient Mixture F-12 Ham without L-glutamine. 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 |
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**INORGANIC SALTS**
Calcium chloride dihydrate | 44.100 |
Copper sulphate pentahydrate | 0.0025 |
Ferric sulphate heptahydrate | 0.834 |
Magnesium chloride anhydrous | 57.650 |
Potassium chloride | 223.600 |
Sodium chloride | 7599.000 |
Sodium phosphate, dibasic anhydrous | 142.040 |
Zinc sulphate tetrahydrate | 0.863 |
**AMINO ACIDS**
Glycine | 7.500 |
L-Alanine | 8.910 |
L-Arginine hydrochloride | 210.700 |
L-Asparagine anhydrous | 15.010 |
L-Aspartic acid | 13.300 |
L-Cysteine hydrochloride | 35.120 |
L-Glutamic acid | 14.700 |
L-Histidine hydrochloride monohydrate | 20.960 |
L-Isoleucine | 3.940 |
L-Leucine | 13.100 |
L-Lysine hydrochloride | 36.500 |
L-Methionine | 4.480 |
L-Phenylalanine | 4.960 |
L-Proline | 34.500 |
L-Serine | 10.500 |
L-Threonine | 11.900 |
L-Tryptophan | 2.040 |
L-Tyrosine disodium salt | 7.810 |
L-Valine | 11.700 |
**VITAMINS**
Biotin | 0.0073 |
Choline chloride | 13.960 |
D-Ca-Pantothenate | 0.480 |
Folic acid | 1.320 |
Nicotinamide | 0.037 |
Pyridoxine hydrochloride | 0.062 |
Riboflavin | 0.038 |
Thiamine hydrochloride | 0.340 |
Vitamin B12 | 1.360 |
i-Inositol | 18.000 |
**OTHERS**
D-Glucose | 1801.600 |
Hypoxanthine sodium salt | 4.770 |
Linoleic acid | 0.084 |
Lipoic acid | 0.210 |
Phenol red sodium salt | 1.240 |
Putrescine dihydrochloride | 0.161 |
Sodium pyruvate | 110.100 |
Thymidine | 0.730 |

Directions:
1. Suspend 10.5gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 1.176gms of sodium bicarbonate powder (TC230) or 15.68ml of 7.5% sodium bicarbonate solution (TCL013) and 0.146gms of L-glutamine (TC243) or 5.0ml of 200mM L-glutamine solution (TCL012) for 1 litre of medium and stir until dissolved.

Please refer disclaimer overleaf
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 ºC 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)
- L-Glutamine powder (TC243)
- L-Glutamine solution 200mM (TCL012)
- Foetal bovine serum (RM1112/RM10432)

Quality Control:

Appearance
Off-white to Creamish white, homogenous powder.

Solubility
Clear solution at 10.5 gms/L.

pH without Sodium Bicarbonate
5.20 - 5.80

pH with Sodium Bicarbonate
7.20 - 7.70

Osmolality without Sodium Bicarbonate
260.00 - 300.00

Osmolality with Sodium Bicarbonate
290.00 - 330.00

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 SEU/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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