

Technical Information

Nutrient Mixture F-12 Ham, Kaighn's Modification With L-Glutamine Without Sodium bicarbonate

Product Code: AT1106

Application: - 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 Mixture 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.

Kaighn's modification of Ham's F-12 is a complex formulation of F-12 with increased amounts of amino acids and pyruvate. Salts used in this formulation are as given by Konisberg. This modification favors the growth and differentiation of rat and chicken cells and primary human liver cells. AT1106 is Nutrient mixture F-12 Ham, Kaighn's modification with 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 / Litre
INORGANIC SALTS	
Calcium chloride dihydrate	135.240
Copper sulphate pentahydrate	0.0025
Disodium hydrogen phosphate	115.020
Ferrous sulphate heptahydrate	0.834
Magnesium chloride anhydrous	49.700
Magnesium sulphate anhydrous	93.700
Potassium chloride	283.290
Potassium dihydrogen phosphate	58.500
Sodium chloride	7597.200
Zinc sulphate heptahydrate	0.1437
AMINO ACIDS	
Glycine	15.010
L-Alanine	17.800
L-Arginine hydrochloride	421.400
L-Asparagine, monohydrate	30.020
L-Aspartic acid	26.620
L-Cysteine hydrochloride heptahydrate	70.240
L-Glutamic acid	29.420
L-Glutamine	292.200
L-Histidine hydrochloride monohydrate	41.920
L-Isoleucine	7.872
L-Leucine	26.240
L-Lysine hydrochloride	73.040
L-Methionine	8.960
L-Phenylalanine	9.920
L-Proline	69.060

L-Serine	21.020
L-Threonine	23.820
L-Tryptophan	4.080
L-Tyrosine Disodium Salt	13.500
L-Valine	23.420
VITAMINS	
Biotin	0.073
Choline chloride	13.960
D-Ca-Pantothenate	0.477
Folic acid	1.320
Nicotinamide	0.037
Pyridoxine hydrochloride	0.061
Riboflavin	0.0376
Thiamine hydrochloride	0.337
Vitamin B12	1.355
i-Inositol	18.020
OTHERS	
D-Glucose	1260.000
Hypoxanthine Sodium Salt	4.083
Lipoic acid	0.2063
Phenol red Sodium Salt	3.318
Putrescine dihydrochloride	0.322
Sodium pyruvate	220.000
Thymidine	0.726

Methodology

1. Suspend 11.1 gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 2.50gms of sodium bicarbonate powder (TC1230) or 33.3ml of 7.5% sodium bicarbonate solution (TCL1013) 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 (TCL1010)
Sodium bicarbonate (TC1230)
Sodium bicarbonate solution, 7.5% (TCL1013)
1N Hydrochloric acid (TCL1003)
1N Sodium hydroxide (TCL1002)
Foetal bovine serum (BA3112/BA30432)

Quality Control

Appearance

Off-white to Creamish white, homogenous powder .

Solubility

Clear solution at 11.1gms/L

pH without Sodium Bicarbonate

5.30-5.90

pH with Sodium Bicarbonate

7.40-8.00

Osmolality without Sodium Bicarbonate

270.00-310.00

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.

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.
- **Central Drug House Pvt. Ltd.** reserves the right to make changes to specifications and information related to the products at any time.
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