CDH

Product Specification

cdhfinechemical.com

Technical Information

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

Product Code: AT1144A

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 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.
AT1144A is Nutrient Mixture F-12 Ham without L-glutamine. phenol red. Users are advised to reviewthe literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

Composition**

Ingredients	mg/Litre
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 monohydrate	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

CDH

Product Specification

cdhfinechemical.com

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
myo-Inositol	18.000
OTHERS	
D-Glucose	1801.600
Hypoxanthine sodium salt	4.770
Linoleic acid	0.084
Lipoic acid	0.210
Putrescine dihydrochloride	0.161
Sodium pyruvate	110.100
Thymidine	0.730

Methodology

1. Suspend 10.5 gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 1.176gms of sodium bicarbonate powder (TC1230) or 15.68ml of 7.5% sodium bicarbonate solution (TCL1013) and 5.0ml of 200mM L-glutamine solution (TCL1012) 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 powder (TC1230)

Sodium bicarbonate solution, 7.5% (TCL1013)

1N Hydrochloric acid (TCL1003)

1N Sodium hydroxide (TCL1002)

L-Glutamine powder (TC1243)

L-Glutamine solution 200mM (TCL1012)

Foetal bovine serum (BA3112/BA30432)

CDH

Product Specification

cdhfinechemical.com

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

7.00 -7.60

Osmolality without Sodium Bicarbonate

250.00 -290.00

Osmolality with Sodium Bicarbonate

280.00 -320.00

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 /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 recommendedsince 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 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.

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.
- Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing of diagnostic reagents extra.
- Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.
- Do not use the products if it fails to meet specifications for identity and performance parameters.