

Nutrient Mixture F-12 Ham, Coon's Modification

Without L-Glutamine and Sodium bicarbonate

Product Code: AT146A

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 Mixture F12, Coon's modification was developed to determine optimal conditions for obtaining viable hybrids by treatment of parental cells with sendai virus. The medium was modified by doubling the concentration of amino acids and pyruvate and adding ascorbic acid.

AT146A is Nutrient Mixture F-12 Ham, Coon's modification 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
INORGANIC SALTS	
Calcium chloride dihydrate	165.000
Copper sulphate pentahydrate	0.0025
Disodium hydrogen phosphate anhydrous	132.040
Ferric sulphate heptahydrate	0.834
Magnesium chloride hexahydrate	106.000
Magnesium sulphate anhydrous	50.800
Potassium chloride	305.000
Potassium dihydrogen phosphate	68.000
Sodium chloride	7530.000
Zinc sulphate heptahydrate	0.144
AMINO ACIDS	
Glycine	16.000
L-Alanine	18.000
L-Arginine hydrochloride	422.000
L-Asparagine anhydrous	30.000
L-Aspartic acid	26.000
L-Cysteine hydrochloride	70.260
L-Glutamic acid	30.000
L-Histidine hydrochloride monohydrate	42.000

L-Isoleucine	7.800
L-Leucine	26.200
L-Lysine hydrochloride	73.000
L-Methionine	9.000
L-Phenylalanine	10.000
L-Proline	70.000
L-Serine	21.000
L-Threonine	23.800
L-Tryptophan	4.000
L-Tyrosine disodium salt	15.860
L-Valine	23.400
VITAMINS	
Biotin	0.0073
Choline chloride	13.960
D-Ca-Pantothenate	0.480
Folic acid	1.320
L-Ascorbic acid	45.000
Nicotinamide	0.040
Pyridoxine hydrochloride	0.060
Riboflavin	0.040
Thiamine hydrochloride	0.340
Vitamin B12	1.360
i-Inositol	18.020
OTHERS	
D-Glucose	2000.000
Hypoxanthine sodium salt	4.040
Linoleic acid	0.090
Lipoic acid	0.206
Phenol red sodium salt	1.240
Putrescine dihydrochloride	0.300
Sodium pyruvate	220.000
Thymidine	0.700

Directions :

1. Suspend 11.6gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 2.676gms of sodium bicarbonate powder (TC230) or 35.68ml of 7.5% sodium bicarbonate solution (TCL013) and 0.292gms of L-glutamine (TC243) or 10.0ml of 200mM L-

glutamine solution (TCL012) 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)

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 11.6gms/L.

pH without Sodium Bicarbonate

4.80 -5.40

pH with Sodium Bicarbonate

7.30 -7.90

Osmolality without Sodium Bicarbonate

270.00 -310.00

Osmolality with Sodium Bicarbonate

330.00 -370.00

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 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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