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Technical Datasheet

Nutrient Mixture F-10 Ham

With L-Glutamine and 25mM HEPES buffer
Without Sodium bicarbonate

Product Code: AT083

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 F10 was designed for clonal growth of CHO cells and chick embryo cells under serum free conditions. It is now widely used for culturing a variety of cells which include human diploid cells and white blood cells for chromosomal analysis and primary explants of rat, rabbit and chicken tissues.

AT083 is Nutrient Mixture F-10 Ham with L-glutamine and 25mM HEPES buffer. HEPES, a zwitterionic buffer having a pKa of 7.3 at 37ºC prevents the initial rise in pH that tends to occur at the initiation of a culture and increases the buffering capacity of the medium. 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	44.100
Copper sulphate pentahydrate	0.0025
Ferric sulphate heptahydrate	0.834
Magnesium sulphate anhydrous	74.640
Potassium chloride	285.000
Potassium phosphate monobasic	83.000
Sodium chloride	7400.000
Sodium phosphate dibasic anhydrous	153.700
Zinc sulphate heptahydrate	0.0288
AMINO ACIDS	
Glycine	7.510
L-Alanine	8.910
L-Arginine hydrochloride	211.000
L-Asparagine monohydrate	15.010
L-Aspartic acid	13.300
L-Cysteine hydrochloride monohydrate	35.130
L-Glutamic acid	14.700
L-Glutamine	146.000
L-Histidine hydrochloride monohydrate	21.000
L-Isoleucine	2.600
L-Leucine	13.100
L-Lysine hydrochloride	29.300
L-Methionine	4.480
L-Phenylalanine	4.960
L-Proline	11.500
L-Serine	10.500
L-Threonine	3.570
L-Tryptophan	0.600
L-Tyrosine disodium salt dihydrate	2.610
L-Valine	3.500
VITAMINS	
Biotin	0.024
Choline chloride	0.698
D-Ca-Pantothenate	0.715
Folic acid	1.320
Nicotinamide	0.615
Pyridoxine hydrochloride	0.206
Riboflavin	0.376
Thiamine hydrochloride	1.000
Vitamin B12	1.360
i-Inositol	0.541
OTHERS	
D-Glucose	1100.000
HEPES Buffer	5958.000
Hypoxanthine Sodium Salt	4.080
Lipoic acid	0.210
Phenol red Sodium Salt	1.300
Sodium pyruvate	110.000
Thymidine	0.730

Directions :

1. Suspend 15.8gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.

2. Add 1.2gms of sodium bicarbonate powder (TC230) or 16.0ml 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)
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 15.8 gms/L.

pH without Sodium Bicarbonate
5.60 -6.20

pH with Sodium Bicarbonate
6.40 -7.00

Osmolality without Sodium Bicarbonate(mOsm/Kg H_2O)
270.00 -310.00

Osmolality with Sodium Bicarbonate(mOsm/Kg H_2O)
300.00 -340.00

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.

Endotoxin Content
NMT 1EU/ml

Storage and Shelf Life:

1. All the powdered media and prepared liquid culture media should be stored at 2-8ºC. 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.