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Technical Datasheet

NCTC 109 Medium

Without L-Glutamine and Sodium bicarbonate

Product Code: AT138A

Product Description :

NCTC 109 is one of the chemically defined medium in the series on NCTC media developed by Virginia Evans of the Tissue Culture Section of National Cancer Institute, Bethesda. NCTC 109 was the result of many years of development and modifications. The medium was originally formulated to establish and maintain a strain of mouse cells, L929. The medium has been shown to support growth of fibroblast-like and epithelial- like cells of both normal and malignant origin from mice, hamsters, monkeys and humans. NCTC 135 is similar to NCTC 109 except that L-Cysteine has been replaced with L-Cystine due to possible side effects of L-Cysteine on certain cell lines.

AT138A is NCTC 109 Medium without L-glutamine and sodium bicarbonate. 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	265.000
Magnesium sulphate anhydrous	100.000
Potassium chloride	400.000
Sodium acetate Anhydrous	30.000
Sodium chloride	6800.000
Sodium dihydrogen phosphate anhydrous	122.000
AMINO ACIDS	
Glycine	13.510
Hydroxy-L-Proline	4.090
L-Alanine	34.480
L-Arginine hydrochloride	31.160
L-Asparagine monohydrate	9.190
L-Aspartic acid	9.910
L-Cysteine hydrochloride monohydrate	289.710
L-Cystine dihydrochloride	13.680
L-Glutamic acid	8.260
L-Histidine hydrochloride monohydrate	26.650
L-Isoleucine	18.040
L-Leucine	20.440
L-Lysine hydrochloride	38.430
L-Methionine	4.440
L-Ornithine hydrochloride	9.410
L-Phenylalanine	16.530
L-Proline	6.130
L-Serine	10.750
L-Threonine	18.930
L-Tryptophan	17.500
L-Tyrosine disodium salt dihydrate	23.700
L-Valine	25.000
VITAMINS	
Calciferol	0.250
Choline chloride	1.250
D-Biotin	0.025
D-Pantothenic acid (hemicalcium)	0.025
DL-Tocopherol phosphate disodium salt	0.025
Folic acid	0.040
L-Ascorbic acid	50.000
Menadione sodium bisulphite	0.125
Nicotinamide	0.0625
Nicotinic acid	0.0625
Pyridoxal hydrochloride	0.0625
Pyridoxine hydrochloride	0.0625
Retinol Acetate	0.250
Riboflavin	0.025
Thiamine hydrochloride	0.025
Vitamin B12	10.000
myo-Inositol	0.125
p-Amino benzoic acid (PABA)	0.125
OTHERS	
2' Deoxyadenosine	10.000
2' Deoxycytidine hydrochloride	10.000
2' Deoxyguanosine hydrochloride	10.000
5'-Methylcytosine hydrochloride	0.100
Cocarboxylase	1.000
Coenzyme A sodium salt	2.500
D-Glucosamine hydrochloride	3.850
D-Glucose	1000.000

D-Glucuronolactone	1.800
Flavin Adenine Dinucleotide Disodium salt	1.000
Glucuronate sodium salt	1.800
Glutathione sodium salt	20.000
L-Amino-n-Butyric acid	5.510
L-Taurine	4.180
Phenol red sodium salt	20.000
Thymidine	10.000
Tween 80	12.500
Uridine triphosphate sodium salt	1.000
ß-NAD	7.000
ß-NADP	1.000

Directions :

1. Suspend 9.5gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 2.2gms of sodium bicarbonate powder (TC230) or 29.3ml of 7.5% sodium bicarbonate solution (TCL013)and 0.135gms of L-Glutamine powder (TC243) or 4.6 ml 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)
L-Glutamine powder (TC243)
L-Glutamine solution 200mM (TCL012) 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 9.5 gms/L.

pH without Sodium Bicarbonate
3.00 -3.60
pH with Sodium Bicarbonate
6.80 -7.40

Osmolality without Sodium Bicarbonate(mOsm/Kg H_2O)
230.00 -270.00

Osmolality with Sodium Bicarbonate(mOsm/Kg H_2O)
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.

Endotoxin Content
NMT 1EU/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. 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.