ProductInformation

NCTC-135 MEDIUM
HYBRI-MAX®
With L-Glutamine, Without Sodium Bicarbonate

Product Number **N5138**
Storage Temperature 2-8°C

Product Description
NCTC-135 was developed by the Tissue Culture Section, Laboratory of Biology, National Cancer Institute (NCI), Bethesda, Maryland. NCTC-135 is a modification of NCTC-109 medium, also developed by the NCI, differing only in that L-cysteine•HCl was replaced with L-cystine due to possible toxic effects of L-cysteine•HCl on certain cell types. NCTC-109 and the 135 modification were formulated to establish and maintain a strain of mouse cells (L929) in a chemically defined and serum-free environment. Successful establishment of L929 cells led to further nutritional and metabolic studies and the establishment of at least ten other cell lines adapted to grow in NCTC-135 medium.

NCTC-135 MEDIUM, Product No. N5138 is one of the cell culture media available from Sigma. The selection of a nutrient medium is strongly influenced by 1] type of cell, 2] type of culture [monolayer, suspension, clonal] and 3] degree of chemical definition necessary. It is important to review the literature for recommendations concerning medium, supplementation and physiological parameters required for a specific cell line.

Components	g/L
Calcium Chloride•2 H_2O	0.265
Magnesium Sulfate (anhydrous)	0.1
Potassium Chloride	0.4
Sodium Acetate (anhydrous)	0.03
Sodium Chloride	6.8
Sodium Phosphate Monobasic (anhydrous)	0.122
L-Alanine	0.03148
L-Arginine•HCl	0.03116
L-Asparagine• H_2O	0.00919
L-Aspartic Acid	0.00991
L-Cystine•2HCl	0.01368
L-Glutamic Acid	0.00826
L-Glutamine	0.13573
Glycine	0.01351
L-Histidine•HCl• H_2O	0.02665
Hydroxy-L-Proline	0.00409
L-Isoleucine	0.01804
L-Leucine	0.02044
L-Lysine•HCl	0.03843
L-Methionine	0.00444
L-Ornithine•HCl	0.00941
L-Phenylalanine	0.01653
L-Proline	0.00613
L-Serine	0.01075
L-Threonine	0.01893
L-Tryptophan	0.0175
L-Tyrosine•2Na•2H_2O	0.0237
L-Valine	0.025
L-Ascorbic Acid•Na	0.05
D-Biotin	0.000025
Calciferol	0.00025
Choline Chloride	0.00125
Folic Acid	0.000025
myo-Inositol	0.000125
Menadione (sodium bisulfite)	0.00004
Niacinamide	0.0000625
Nicotinic Acid	0.0000625
p-Amino Benzoic Acid	0.000125
D-Panthothenic Acid (hemicalcium)	0.000025
Pyridoxal•HCl	0.0000625
Pyridoxine•HCl	0.0000625
Retinol Acetate	0.00025
Riboflavin	0.000025
Thiamine•HCl	0.000025
DL- -Tocopherol Phosphate•2Na	0.000025
Vitamin B-12	0.01
L-Amino-n-Butyric Acid	0.00551
Cocarboxylase	0.001
Coenzyme A•Na	0.0025
2'-Deoxyadenosine	0.01
2'-Deoxycytidine•HCl	0.01
2'-Deoxyguanosine	0.01
Flavin Adenine Dinucleotide•2Na	0.001
D-Glucosamine•HCl	0.00385
D-Glucose	1.0
Glucuronate•Na	0.0018
D-Glucuronolactone	0.0018
Glutathione•Na	0.02
5'-Methylcytosine•HCl	0.0001
β-NAD	0.007
β-NADP•Na	0.001
Phenol Red•Na	0.02
Taurine	0.00418
Thymidine	0.01
TWEEN 80	0.0125
Uridine 5'-Triphosphate•Na	0.001

Precautions and Disclaimer
REAGENT
For In Vitro Diagnostic Use

Preparation Instructions
Powdered media are extremely hygroscopic and should be protected from atmospheric moisture. The entire contents of each package should be used immediately after opening.

Preparing a concentrated solution of medium is not recommended as precipitates may form.

Supplements can be added prior to filtration or introduced aseptically to sterile medium. The nature of the supplement may affect storage conditions and shelf life of the medium.

1. Measure out 90% of final required volume of water. Water temperature should be 15-20°C.
2. While gently stirring the water, add the powdered medium. Stir until dissolved. Do NOT heat.
3. Rinse original package with a small amount of water to remove all traces of powder. Add to solution in step 2.
4. To the solution in step 3, add 2.2 g sodium bicarbonate or 29.3 ml of sodium bicarbonate solution [7.5%w/v] for each liter of final volume of medium being prepared. Stir until dissolved.
5. While stirring, adjust the pH of the medium to 0.1-0.3 pH units below the desired pH since it may rise during filtration. The use of 1N HCl or 1N NaOH is recommended.
6. Add additional water to bring the solution to final volume.
7. Sterilize immediately by filtration using a membrane with a porosity of 0.22 microns.
8. Aseptically dispense medium into sterile container.

Storage/Stability
Store the dry powdered medium at 2-8°C under dry conditions and liquid medium at 2-8°C in the dark. Deterioration of the powdered medium may be recognized by any or all of the following: [1] color change, [2] granulation/clumping, [3] insolubility. Deterioration of the liquid medium may be recognized by any or all of the following: [1] pH change, [2] precipitate or particulate matter throughout the solution, [3] cloudy appearance [4] color change. The nature of supplements added may affect storage conditions and shelf life of the medium. Product label bears expiration date.

Procedure
Materials Required but Not Provided
Water for tissue culture use [W3500]
Sodium Bicarbonate [S5761] or
Sodium Bicarbonate Solution, 7.5% [S8761]
1N Hydrochloric Acid [H9892]
1N Sodium Hydroxide [S2770]
Medium additives as required

Product Profile

Appearance	off-white powder
Moisture content	≤2.0%
Solubility	clear solution at 1x concentration
pH at RT [without sodium bicarbonate]	5.2 ± 0.3
pH at RT [with sodium bicarbonate]	7.5 ± 0.3
Osmolality [without sodium bicarbonate]	250 mOsm/kg H_2O ± 5%
Osmolality [with sodium bicarbonate]	290 mOsm/kg H_2O ± 5%
Endotoxin	≤0.5 EU/ml at 1x
Amino Acid Analysis by HPLC	Analysis has confirmed that amino acids are present at concentrations consistent with the formula.
Key Element Analysis by ICAP	Analysis has confirmed that key elements are present at concentrations consistent with the formula.

Biological Performance Characteristics
Biological performance is assessed using an appropriate cell line(s). Growth studies are carried through 2 subculture generations. Cells are counted and growth is plotted as a logarithmic function of time in culture. Seeding efficiencies, doubling time, and final cell densities are determined. During the testing period cultures are examined microscopically for atypical morphology and evidence of cytotoxicity. This product is also assessed for its ability to support clonal growth and maintenance of hybridoma cells. Test results are available upon request.

References
1. Evans, V.J. et al. (1964). Chemically defined media for cultivation of long-term cell strains from four mammalian species. Exp. Cell Res. 36, 439.

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