NCTC-135 MEDIUM
With L-Glutamine, Without Sodium Bicarbonate
Product Number **N3262**

Product Description
NCTC-135 was developed by the Tissue Culture Section, Laboratory of Biology, National Cancer Institute (NCI), Bethesda, Maryland. 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.

Components	**g/L**
Calcium Chloride (anhydrous)	0.2
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•2H2O	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 R&D use only. Not for drug, household or other uses.

Preparation Instructions
Powdered media are hygroscopic and should be protected from 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.

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 and 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 particulates, [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

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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