MCDB 153 Medium

With Trace elements, L- Glutamine and 28mM HEPES buffer
Without Sodium bicarbonate

Product Code: AT135

Product Description :

MCDB media were developed for the culture of specific cell types without a serum supplement. The media were supplemented with growth factors, hormones, trace elements, or low levels of dialyzed fetal bovine serum protein (FBSP). Each MCDB medium was formulated for a specific cell type. MCDB 105 and 110 were formulated for rapid clonal growth normal of human diploid cells. MCDB 131 medium was originally developed for the clonal growth of human micro-vascular endothelial cells (HMVEC). MCDB 151, 201 and 302 were originally developed for human keratinocytes, clonal growth of chick embryo fibroblasts and CHO cells.

AT135 is MCDB 153 with trace elements, L-glutamine and 28mM 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	
Ammonium metavanadate	0.000585
Calcium chloride dihydrate	4.411
Cupric sulphate pentahydrate	0.00275
Disodium hydrogen phosphate anhydrous	284.088
Ferrous sulphate heptahydrate	1.390
Magnesium chloride hexahydrate	122.000
Manganese sulphate	0.000151
Molybdic acid ammonium tetrahydrate	0.00124
Nickel chloride	0.00012
Potassium chloride	111.830
Sodium acetate anhydrous	301.530
Sodium chloride	7599.000
Sodium metasillicate nonahydrate	0.1421
Sodium selenite	0.0038
Stannous chloride monohydrate	0.000113
Zinc sulphate heptahydrate	0.144
AMINO ACIDS	
Glycine	7.510
L-Alanine	8.910
L-Arginine hydrochloride	210.700
L-Asparagine monohydrate	15.000
L-Aspartic acid	3.990
L-Cysteine hydrochloride monohydrate	42.040
L-Glutamic acid	14.710
L-Glutamine	877.200
L-Histidine hydrochloride monohydrate	16.770
L-Isoleucine	1.968
L-Leucine	65.600
L-Lysine hydrochloride	18.270
L-Methionine	4.480
L-Phenylalanine	4.960
L-Proline	34.530
L-Serine	63.060
L-Threonine	11.910
L-Tryptophan	3.060
L-Tyrosine disodium salt dihydrate	3.410
L-Valine	35.130
VITAMINS	
Choline chloride	13.960
D-Biotin	0.0146
D-Pantothenic acid (hemicalcium)	0.238
Folic acid	0.790
Niacinamide	0.03663
Pyridoxine hydrochloride	0.06171
Riboflavin	0.0376
Thiamine hydrochloride	0.337
Vitamin B12	0.407
myo-Inositol	18.020
OTHERS	
Adenine hydrochloride	30.880
D-Glucose	1081.000
HEPES buffer	6600.000
Phenol red sodium salt	1.242
Putrescine dihydrochloride	0.161
Sodium pyruvate	55.000

Thioctic acid	0.206
Thymidine	0.727

Directions :

1. Suspend 17.7gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 1.176gms of sodium bicarbonate powder (TC230) or 15.7ml 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 powder (TC230)
Sodium bicarbonate solution, 7.5% (TCL013)
1N Hydrochloric acid (TCL003)
1N Sodium hydroxide (TCL002)
Foetal bovine serum (RM1112/RM10432)

Quality Control:

Appearance
Off-white to Creamish white, homogenous powder.

Solubility
Clear solution at 17.7 gms/L.

pH without Sodium Bicarbonate
5.10 -5.70

pH with Sodium Bicarbonate
6.40 -7.00

Osmolality without Sodium Bicarbonate
300.00 -340.00

Osmolality with Sodium Bicarbonate
320.00 -360.00

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 or after 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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