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Technical Datasheet

CMRL 1066 Medium

Without L-Glutamine, Phenol red and Sodium bicarbonate

Product Code: AT226A

Product Description :

CMRL media are based on the composition of Medium 199. Nucleic acid derivatives, coenzymes omitted in H597 modification were retained in CMRL 1066. CMRL 1066 is a chemically defined medium formulated for long term cultivation of mouse L cells in the absence of protein supplement. Although originally designed for use in serum free conditions, this medium supports growth of a variety of cell lines in the presence of serum.

AT226A is CMRL 1066 Medium without L-glutamine and phenol red 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	97.690
Potassium chloride	400.000
Sodium acetate anhydrous	50.000
Sodium chloride	6800.000
Sodium dihydrogen phosphate anhydrous	122.000
AMINO ACIDS	
Glycine	50.000
L-Alanine	25.000
L-Arginine	57.870
L-Aspartic acid	30.000
L-Cysteine hydrochloride monohydrate	260.000
L-Cystine	20.000
L-Glutamic acid	75.000
L-Histidine hydrochloride monohydrate	20.000
L-Isoleucine	20.000
L-Leucine	60.000
L-Lysine hydrochloride	70.000
L-Methionine	15.000
L-Phenylalanine	25.000
L-Proline	40.000
L-Serine	25.000
L-Threonine	30.000
L-Tryptophan	10.000
L-Tyrosine disodium salt dihydrate	40.000
L-Valine	25.000
Trans-4-Hydroxy-L-Proline	10.000
VITAMINS	
Choline chloride	0.500
D-Biotin	0.010
D-Pantothenate (Hemicalcium)	0.010
Folic acid	0.010
L-Ascorbic acid sodium salt	50.000
Nicotinamide	0.025
Nicotinic acid	0.025
Pyridoxal hydrochloride	0.025
Pyridoxine hydrochloride	0.025
Riboflavin	0.010
Thiamine hydrochloride	0.010
myo-Inositol	0.050
p-Amino benzoic acid (PABA)	0.050
OTHERS	
2' Deoxyadenosine	10.000
2' Deoxycytidine hydrochloride	11.600
2' Deoxyguanosine	10.000
5-Methyldeoxycytidine	0.100
Cholesterol	0.200
Cocarboxylase	1.000
Coenzyme A sodium salt	2.500
D-Glucose	1000.000
D-Glucuronic acid sodium salt	3.880
FAD disodium salt	0.106
Glutathione reduced	10.000
Thymidine	10.000
Tween 80	5.000
Uridine-5-Triphosphate.Na	1.000
β-NAD	7.000
β-NADP sodium salt	1.000

Directions :

1. Suspend 9.8gms 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 0.1gms of L-glutamine powder (TC243) or 3.42ml 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 200mM solution (TCL012)
1N Hydrochloric acid (TCL003)
1N Sodium hydroxide (TCL002)
Fetal bovine serum (RM1112)

Quality Control:

Appearance
Off-white to Creamish white, homogenous powder.

Solubility
Clear solution at 9.8gms/L.

pH without Sodium Bicarbonate
3.20 -3.80

pH with Sodium Bicarbonate
6.80 -7.40

Osmolality without Sodium Bicarbonate(mOsm/Kg H_2O)
240.00 -280.00

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 2-8°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.