D-22 Insect Medium

Without Sodium bicarbonate

Product Code: IM005

Product Description :

D-22 medium (1976) is the final modification of the original D20 medium developed by Echalier and Ohanessian (1970). The medium composition closely initiates the principal characteristics of the body fluid of Drosophila 3rd instar larvae. This medium has been extensively used to grow many established Drosophila cell lines, including the famous Kc cell line.

IM005 is D-22 medium. It is a mixture of inorganic anions like sodium and potassium glutamate, glycine and Kreb's cycle intermediates. The medium also contains lactalbumin hydrolysate which provides large number of free amino acids. The medium is further made nutritionally rich by yeast extract and vitamins. Supplemented with 5 -20% fetal bovine serum, this medium can be used for the maintenance of primary and established cell lines desired from Drosophila melanogaster. 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	1059.460
Magnesium chloride anhydrous	421.654
Magnesium sulphate anhydrous	1641.350
Sodium acetate anhydrous	13.868
Sodium dihydrogen phosphate anhydrous	330.769
AMINO ACIDS	
Glycine	5000.000
Potassium glutamate monohydrate	4975.000
Sodium glutamate monohydrate	7980.000
VITAMINS	
Choline chloride	0.200
D-Biotin	0.010
D-Ca-Pantothenate	0.020
Folic acid	0.020
Niacin	0.020
Pyridoxine hydrochloride	0.020
Riboflavin	0.020
Thiamine hydrochloride	0.020
myo-Inositol	0.020
p-Amino benzoic acid (PABA)	0.020
OTHERS	
D-Glucose	1800.000
L-Malic acid	600.000
Lactalbumin hydrolysate	13600.000
Succinic acid disodium anhydrous	32.990
Yeast extract	1360.000

Directions :

1. Suspend 38.8gms in 900ml tissue culture grade water with constant, gentle stirring.
2. Adjust the pH of the final medium to 6.7 using 1N NaOH or 1N KOH.
3. Adjust the osmolality to 340 - 360mOsm/kg H2O. Osmolality can be increased by 10mOsm/kg H2O by adding 0.3gms per litre of sodium chloride (TC046) or 0.4gms per litre of potassium chloride (TC010). Osmolality can be decreased by 10mOsm/kg H2O by adding 27.8ml of water to per litre of the medium.
4. Sterilize immediately by filtration using a membrane with porosity of 0.22µ or less.
5. Aseptically, dispense medium into sterile container.
6. Store liquid medium at 2-8°C and in dark till use.

Material required but not provided :

Tissue culture grade water (TCL010)
1N Hydrochloric acid (TCL003)
1N Sodium hydroxide (TCL002)
Sodium chloride (TC046)
Potassium chloride (TC010)
Fetal bovine serum (RM1112/ RM10432)

Quality Control:

Appearance

Off-white to creamish white, homogenous powder

Solubility

Clear solution at 38.8gms/L.

pH without Sodium Bicarbonate

5.40 -6.00

Osmolality without Sodium Bicarbonate

310.00 -350.00

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. 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 of the prepared medium is a critical factor 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 and 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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