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Product Specification

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Technical Information

Grace's Insect Medium

w/ L- Glutamine w/o Sodium bicarbonate

Product Code: IM1001

Application:- Grace's medium is a modification of the original formula developed by Wyatt. Grace (1962) improved the medium by introducing ten water soluble vitamins in Wyatt's formula. Grace successfully established four strains of cells from ovarian tissue of Australian emperor gum moth using this medium. These were the first continuous cell lines developed from insect tissue.
IM1001 is Grace's Insect Medium with L-glutamine. It can be used to culture cells derived from a variety of insects especially Lepidopterans and some species of Dipterans. Originally the basal medium was supplemented with plasma from insects, the tissues of which were cultured or plasma from silkworm, Bombyx mori. Presently, the medium is supplemented with 5- 20% fetal bovine serum. Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

Composition**

Ingredients	mg/Litre
INORGANIC SALTS	
Calcium chloride dehydrate	1320.000
Magnesium chloride anhydrous	1068.200
Magnesium sulphate anhydrous	1356.650
Potassium chloride	2240.000
Sodium phosphate monobasic monohydrate	1007.000
AMINO ACIDS	
DL-Serine	1100.000
Glycine	650.000
L-Alanine	225.000
L-Arginine hydrochloride	700.000
L-Asparagine monohydrate	400.000
L-Aspartic acid	350.000
L-Cystine dihydrochloride	25.000
L-Glutamic acid	600.000
L-Glutamine	600.000
L-Histidine hydrochloride monohydrate	3376.000
L-Isoleucine	50.000
L-Leucine	75.000
L-Lysine hydrochloride	625.000
L-Methionine	50.000
L-Phenylalanine	150.000
L-Proline	350.000
L-Threonine	175.000
L-Tryptophan	100.000
L-Tyrosine disodium salt	72.000
L-Valine	100.000
ß-Alanine	200.000

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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	
Alpha-Ketoglutaric acid D(+)	370.000
Glucose	700.000
D-Fructose	400.000
Fumaric acid, free acid	55.000
L-Malic acid, free acid	670.000
Succinic acid	60.000
Sucrose	26680.000

Methodology

1. Suspend 45.9gms in 900ml tissue culture grade water with constant, gentle stirring until the medium is completely dissolved. Do not heat the water.
2. Add 0.35gms of sodium bicarbonate powder (TC1230) or 4.7ml of 7.5% of sodium bicarbonate solution (TCL1013) for each litre of the medium. Stir until dissolved.
3. Adjust the pH to 6.2 using 1N KOH.
4. Make up the final volume to 1000ml.
5. Adjust the Osmolality as desired. For Lepidopterans cell line, osmolality of 340 - 360mOsm/KgH_2O is recommended. The osmolality can be increased by 10mOsm/KgH_2O by adding 0.4gms of potassium chloride (TC1010) or 0.3gms of sodium chloride (TC1046) to each litre of the medium. Osmolality can be decreased by 10mOsm/KgH_2O by adding 27.8ml of water to per litre of medium.
6. Sterilize the medium using a membrane filter with porosity of 0.22 microns or less.
7. Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into sterile containers.
8. Store liquid medium at 2-8°C and in dark till use.

Material required but not provided

Tissue culture grade water (TCL1010)
Sodium bicarbonate (TC1230)
Sodium bicarbonate solution, 7.5% (TCL1013)
1N Hydrochloric acid (TCL1003)
1N Sodium hydroxide (TCL1002)
Fetal bovine serum (BA3112/ BA12432)
Sodium chloride (TC1046)
Potassium chloride (TC1010)

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Quality control

Appearance
Off-white to creamish white, homogenous powder
Solubility
Solution with few particles at 45.9gms/L
pH without Sodium Bicarbonate
3.70 -4.30
pH with Sodium Bicarbonate
3.90 -4.50
Osmolality without Sodium Bicarbonate (mOsm/Kg H_2O)
330.00 -370.00
Osmolality with Sodium Bicarbonate (mOsm/Kg H_2O)
340.00 -380.00
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. 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.
3. 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.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conforms solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate.
- **Central Drug House Pvt. Ltd.** reserves the right to make changes to specifications and information related to the products at any time.
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- Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.
- Do not use the products if it fails to meet specifications for identity and performance parameters.