Grace's Insect Medium
With L-Glutamine
Without Sodium bicarbonate

Product Code: IM001

Product Description:
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.

IM001 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/L
INORGANIC SALTS
Calcium chloride dihydrate 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
β-Alanine 200.000

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
Alpha-Ketoglutaric acid 370.000
D(+) 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

Directions:
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 (TC230) or 4.7ml of 7.5% of sodium bicarbonate solution (TCL013) 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/KgH2O is recommended. The osmolality can be increased by 10mOsm/KgH2O by adding 0.4gms of potassium chloride (TC010) or 0.3gms of sodium chloride (TC046) to each litre of the medium. Osmolality can be decreased by 10mOsm/KgH2O 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 (TCL010)
- Sodium bicarbonate (TC230)
- Sodium bicarbonate solution, 7.5% (TCL013)
- 1N Hydrochloric acid (TCL003)
- 1N Sodium hydroxide (TCL002)
- Fetal bovine serum (RM1112/RM10432)
- Sodium chloride (TC046)
- Potassium chloride (TC010)

**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**
330.00 - 370.00

**Osmolality with Sodium Bicarbonate**
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 SEU/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. Insipite 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. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. 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.

HiMedia Laboratories Pvt. Ltd. A-516, Swastik Disha Business Park, Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. Customer care No.: 022-6147 1919 Email: info@himedialabs.com Website: www.himedialabs.com