

### Technical Information

#### Mitsubishi and Maramorosch Insect Medium Without Sodium bicarbonate

#### Product Code: IM1002

**Application:-** Mitsubishi and Maramorosch Insect Medium is specially used for the growth and propagation of mosquito cell lines especially *Aedes aegypticus*. It was originally developed to grow cells derived from Leafhopper. Subsequently, it has been used to culture cells derived from a number of insect species.

IM1002 is Mitsubishi and Maramorosch Insect Medium. It needs to be supplemented with 5-20 % fetal bovine serum. Lactalbumin hydrolysate serves as a source of free amino acid, whereas yeast extract serves as a source of vitamins. When supplemented with fetal bovine serum, this medium is most commonly used to culture cells derived from a number of mosquitoes. 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
<b>INORGANIC SALTS</b>	
Calcium chloride dehydrate	190.000
Magnesium chloride anhydrous	46.900
Potassium chloride	200.000
Sodium chloride	7000.000
Sodium phosphate monobasic	173.900
<b>OTHERS</b>	
D(+) Glucose	4000.000
Lactalbumin hydrolysate	6500.000
Yeast extract	5000.000

#### Methodology

1. Suspend 23.1gms in 900ml tissue culture grade water with constant, gentle stirring until the medium is completely dissolved. Do not heat the water.
2. Add 0.12gms of sodium bicarbonate (TC1230) or 1.6ml of 7.5% of sodium bicarbonate solution (TCL1013) for each litre of the medium. Stir until dissolved.
3. Adjust the pH to 0.1- to 0.3 units below the desired range using 1N HCl and 1N NaOH, as pH tends to rise during filtration using 2N KOH. Make up the final volume to 1000ml.
4. Sterilize the medium using a membrane filter with porosity of 0.22 microns or less.
5. Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into sterile containers.
6. Store liquid medium at 15-30°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)
- Foetal bovine serum (BA3112/ BA12432)

### Quality control

#### Appearance

Off-white to creamish white, homogenous powder

#### Solubility

Clear solution at 23.1gms/L.

#### pH without Sodium Bicarbonate

6.20 -6.80

#### pH with Sodium Bicarbonate

6.30 -6.90

#### Osmolality without Sodium Bicarbonate

300.00 -340.00

#### Osmolality with 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 10 EU/ml

### Storage and Shelf Life

1. Mitsubishi and Maramorosch Insect medium should be stored at 15-30°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. 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.
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