

### Technical Information

#### TC-100 Insect Medium w/ L- Glutamine w/o Sodium bicarbonate

#### Product Code: IM1007

**Application:-** TC-100 is a modification done by Dr.Gardiner and Dr.Stockdale of the original formulation of Grace's insect medium. Sucrose, fructose and several Kreb's cycle intermediates were omitted from the original formula. The modification was done to optimise the production of Autographia californica NPV virions by cells from the fall armyworm, Spodoptera frugiperda.

IM1007 is TC-100 Insect Medium with L-glutamine. TC-100 is a fully defined medium and does not require supplementation with insect hemolymph. Instead, tryptose broth and fetal bovine serum provide the necessary growth factors . This medium supports the growth of several lepidopteran cell lines. Users are advised to review the literature for recommendations and physiological growth requirements for different cell lines.

#### Composition\*\*

Ingredients	mg/Litre
<b>INORGANIC SALTS</b>	
Calcium chloride dehydrate	1319.820
Magnesium chloride anhydrous	1068.200
Magnesium sulphate anhydrous	1357.630
Potassium chloride	2870.000
Sodium phosphate monobasic	876.920
<b>AMINO ACIDS</b>	
Glycine	650.000
L-Alanine	225.000
L-Arginine hydrochloride	700.000
L-Asparagine	350.000
L-Aspartic acid	350.000
L-Cystine hydrochloride	25.000
L-Glutamic acid	600.000
L-Glutamine	600.000
L-Histidine hydrochloride	3086.340
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-Serine	550.000
L-Threonine	175.000
L-Tryptophan	100.000
L-Tyrosine disodium salt	62.090
L-Valine	100.000

### 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	0.520
myo-Inositol	0.020
p-Amino benzoic acid (PABA)	0.020

### OTHERS

D(+) Glucose	1000.000
Tryptose Broth	2600.000

## Methodology

1. Suspend 20.0gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 0.35gms sodium bicarbonate powder (TC1230) or 4.7ml of 7.5% of sodium bicarbonate solution (TCL1013) for each litre of medium. Stir until dissolved.
3. Adjust the pH to 6.2 using 1N KOH. Use of NaOH may cause precipitation.
4. Make up the final volume to 1000ml.
5. Adjust the Osmolality as desired. For Lepidopterans cell line, osmolality of 340 - 360mOsm/KgH<sub>2</sub>O is recommended. The osmolality can be increased by 10mOsm/KgH<sub>2</sub>O 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<sub>2</sub>O by adding 27.8ml of water to per litre of medium.
6. Sterilize the medium using a membrane filter with porosity of 0.22microns or less.
7. Aseptically dispense the medium in sterile containers.
8. Store liquid medium at 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)  
 Sodium chloride (TC1046)  
 Potassium chloride (TC1010)  
 Foetal bovine serum (BA3112/ BA12432)

## Quality control

### Appearance

Off-white to Creamish white, homogenous powder.

### Solubility

Clear solution at 20.0gms/L.

### pH without Sodium Bicarbonate

3.90 -4.50

### pH with Sodium Bicarbonate

4.70 -5.30

### Osmolality without Sodium Bicarbonate

220.00 -260.00

### Osmolality with Sodium Bicarbonate

230.00 -270.00

### 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. 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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