



TC-100 Insect Medium

With L-Glutamine

Without Sodium bicarbonate

Product Code: IM007

Product Description:

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 Krebs'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.

IM007 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/L |
|------------------------------|----------|
| INORGANIC SALTS | |
| Calcium chloride dihydrate | 1319.820 |
| Magnesium chloride anhydrous | 1068.200 |
| Magnesium sulphate anhydrous | 1357.630 |
| Potassium chloride | 2870.000 |
| Sodium phosphate monobasic | 876.920 |
| AMINO ACIDS | |
| 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 |

Directions:

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 (TC230) or 4.7ml of 7.5% of sodium bicarbonate solution (TCL013) 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/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 0.22microns 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 (TCL010)
Sodium bicarbonate (TC230)
Sodium bicarbonate solution, 7.5% (TCL013)
1N Hydrochloric acid (TCL003)
1N Sodium hydroxide (TCL002)
Sodium chloride (TC046)
Potassium chloride (TC010)
Foetal bovine serum (RM1112/ RM10432)

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 :

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