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Product Specification

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Technical Information

Schneider's Insect Medium

With L-Glutamine

Without Calcium Chloride and Sodium bicarbonate

Product Code: IM1003

Application:- Schneider's insect medium has been specially formulated for the in vitro culture of *Drosophila melanogaster* cells and tissues. A number of cell lines derived from *Drosophila melanogaster* are now available and are extensively used in genetic and molecular biology research. *Drosophila* cells are also used to study recombinant protein expression.
IM1003 is Schneider's insect medium with L-glutamine. It does not contain calcium chloride and sodium bicarbonate, hence needs to be supplemented with calcium chloride and sodium bicarbonate while preparing the complete medium. The basal medium also needs to be supplemented with 5 - 20% of heat inactivated fetal bovine serum to supply the necessary growth factors to the insect cells. Schneider's insect medium is an extremely nutritious medium and supports rapid growth of cells derived from *Drosophila*. It can also be used to culture cell lines derived from other dipteran species. 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	
Disodium hydrogen phosphate	700.000
Magnesium sulphate heptahydrate	3700.000
Potassium chloride	1600.000
Potassium dihydrogen phosphate	450.000
Sodium chloride	2100.000
AMINO ACIDS	
Glycine	250.000
L-Arginine hydrochloride	483.810
L-Aspartic acid	400.000
L-Cysteine hydrochloride	78.000
L-Cystine dihydrochloride	100.000
L-Glutamic acid	800.000
L-Glutamine	1800.000
L-Histidine hydrochloride monohydrate	540.000
L-Isoleucine	150.000
L-Leucine	150.000
L-Lysine hydrochloride	1650.000
L-Methionine	800.000
L-Phenylalanine	150.000
L-Proline	1700.000
L-Serine	250.000
L-Threonine	350.000
L-Tryptophan	100.000
L-Tyrosine disodium salt	620.000
L-Valine	300.000
ß-Alanine	500.000
OTHERS	
Alpha-Ketoglutaric acid	200.000
D(+) Glucose	2000.000

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D(+)-Trehalose	2000.000
Fumaric acid	100.000
L-Malic acid	100.000
Succinic acid	100.000
Yeast extract	2000.000

Methodology

1. Suspend 26.2gms in 900ml tissue culture grade water with constant, gentle stirring. Material will not go completely in the solution. Do not heat the water.
2. Add 0.4gms of sodium bicarbonate powder (TC1230) or 5.3ml of 7.5% sodium bicarbonate solution (TCL1013) for each litre of the final volume of the medium being prepared. Stir until dissolved.
3. Adjust the pH to 9.2 ± 0.2 with 1N sodium hydroxide with constant stirring. Solution may become turbid.
4. Adjust the pH to 6.7 ± 0.2 with 1N HCl, with constant stirring. Solution will become clear.
5. Add 0.6gms of anhydrous calcium chloride per litre of the medium. Prepare a solution by dissolving 0.6gms of anhydrous calcium chloride (TC1097) in 50ml of tissue culture grade water. Add the solution slowly with constant stirring to avoid precipitate formation.
6. Adjust the pH of the medium to 0.1-0.3 pH units using 1N NaOH or 1N HCl below the desired pH since it tends to rise during filtration. Make up the final volume to 1litre.
7. Adjust the osmolality to 340 - 360mOsm/kg H_2O. Osmolality can be increased by 10mOsm/kg H_2O by adding 0.3gms per litre of 0.4gms per litre of potassium chloride (TC1010) or sodium chloride (TC1046). Osmolality can be decreased by 10mOsm/kg H_2O by adding 27.8ml of water to per litre of the medium.
8. Sterilize immediately by filtration using a membrane with porosity of 0.22 microns or less.
9. Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into sterile containers.
10. 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)
Sodium chloride (TC1046)
Calcium chloride anhydrous (TC1097)
Potassium chloride (TC1010)
Fetal bovine serum (BA3112/ BA12432)

Quality control

Appearance
Off-white to creamish white, homogenous powder
Solubility
Clear solution at 26.2gms/L.
pH without Sodium Bicarbonate
4.20 -4.80
pH with Sodium Bicarbonate
4.80 -5.40
Osmolality without Sodium Bicarbonate
260.00 -300.00
Osmolality with Sodium Bicarbonate
270.00 -310.00
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 three subcultures.
Endotoxin Content
NMT 15EU/ml

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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. Preparation of concentrated medium is not recommended since free base amino acids and salt complexes having low solubility may precipitate in concentrated medium.
3. 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.
4. 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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- Do not use the products if it fails to meet specifications for identity and performance parameters.