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Product Specification

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Technical Information

Sheilds and Sang M3 Insect Medium

w/ L- Glutamine w/o Potassium bicarbonate

Product Code: IM1004

Application:- Sheilds and Sang M3 medium is based on the original formula of D22 medium. The medium supports the growth of the cells derived from *Drosophila melanogaster*. Sheilds and Sang (1977) eliminated chloride salts present in the previous formulation and introduced potassium and sodium ions in the form of glutamate salts. They replaced lactabumin hydrolysate with individual amino acids and retained yeast extract. The formulation also includes Bis-Tris, an inorganic buffer which prevents shifts in pH.
IM1004 is Sheilds and Sangs M3 Medium with Lglutamine. It does not contain potassium bicarbonate. It needs to be supplemented with 10% fetal bovine serum. Although many variations of Shields and Sangs medium have been published, classical Sheilds and Sangs M3 (1977) medium is widely used for growth and maintenance of various *Drosophila* cell lines. 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	
Calcium chloride dehydrate	1006.840
Magnesium sulphate anhydrous	2148.770
Sodium phosphate monobasic	880.000
AMINO ACIDS	
Glycine	500.000
L-Alanine	1500.000
L-Arginine hydrochloride	603.030
L-Asparagine monohydrate	340.710
L-Aspartic acid	300.000
L-Cysteine hydrochloride	200.000
L-Glutamic acid potassium salt	7880.000
L-Glutamic acid sodium salt	6530.000
L-Glutamine	600.000
L-Histidine hydrochloride	681.200
L-Isoleucine	250.000
L-Leucine	400.000
L-Lysine hydrochloride	850.000
L-Methionine	250.000
L-Phenylalanine	250.000
L-Serine	350.000
L-Threonine	500.000
L-Tryptophan	100.000
L-Tyrosine disodium salt	310.380
L-Valine	400.000
Proline	400.000
ß - Alanine	250.000
VITAMINS	
Choline chloride	0.200

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OTHERS	
Bis-Tris	1050.000
D(+) Glucose	10000.000
Oxalacetic acid	250.000
Yeast extract	1000.000

Methodology

1. Suspend 39.8gms in 900ml tissue culture grade water with constant, gentle stirring until the medium is completely dissolved. Do not heat the water.
2. Add 0.5gms of potassium bicarbonate powder or 6.67ml of 7.5% of potassium bicarbonate solution (TCL1024) for each litre of the medium. Stir until dissolved.
3. Adjust the pH to 6.7 using 1N KOH. Make up the final volume to 1000ml.
4. 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 sodium chloride (TC1046) or 0.4gms per litre of potassium chloride (TC1010). Osmolality can be decreased by 10mOsm/kg H_2O by adding 27.8ml of water to per litre of the medium.
5. Sterilize the medium using a membrane filter with porosity of 0.22 microns or less.
6. Aseptically dispense the medium in sterile containers.
7. Store liquid medium at 2-8°C and in dark till use.

Material required but not provided

Tissue culture grade water (TCL1010)
Potassium bicarbonate (BA3789)
Potassium bicarbonate solution, 7.5% (TCL1024)
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 39.8gms/L.
pH without Sodium Bicarbonate
5.50 -6.10
pH with Sodium Bicarbonate
5.80 -6.40
Osmolality without Sodium Bicarbonate
320.00 -360.00
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 5EU/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 potassium 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 bot volume of carbon dioxide is released. Therefore, optimal conditions of pH, potassium 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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