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Product Specification

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Technical Information

TNM-FH Insect Medium

With L-Glutamine, Lactalbumin hydrolysate and Yeast Extract
Without Sodium bicarbonate

Product Code: IM1008

Application:- TNM-FH medium is a modification of Grace's insect medium. The modification was developed by Dr. W. F. Hink to grow Cabbage looper cell line TN-368. Presently, this medium is used to grow cells derived from a variety of lepidopterans species.
IM1008, TNM-FH insect medium, is a modification of Grace's medium supplemented with lactalbumin hydrolysate and yeast extract. L-Glutamine and Lactalbumin hydrolysate provides a large number of free amino acids. Yeast extract serves as a source of vitamins. Supplemented with 5- 20% fetal bovine serum this medium supports growth of number of insect cell lines derived from Lepidopteran 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	
Calcium chloride dehydrate	1324.620
Magnesium chloride anhydrous	1068.200
Magnesium sulphate anhydrous	1357.660
Potassium chloride	2240.000
Sodium dihydrogen phosphate anhydrous	876.920
AMINO ACIDS	
DL-Serine	1100.000
Glycine	650.000
L-Alanine	225.000
L-Arginine hydrochloride	700.000
L-Asparagine monohydrate	350.000
L-Aspartic acid	350.000
L-Cystine dihydrochloride	25.000
L-Glutamic acid	600.000
L-Glutamine	600.000
L-Histidine hydrochloride monohydrate	3377.640
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-Threonine	175.000
L-Tryptophan	100.000
L-Tyrosine disodium salt dehydrate	72.000
L-Valine	100.000
ß-Alanine	200.000

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VITAMINS	
Choline chloride	0.200
D-Biotin	0.010
D-Calcium-Pantothenate	0.020
Folic acid	0.020
Niacin	0.020
Pyridoxine hydrochloride	0.020
Riboflavin	0.020
Thiamine hydrochloride	0.020
myo-Inositol	0.020
p-Amino benzoic acid (PABA)	0.020
OTHERS	
Alpha-Ketoglutaric acid D(+)	370.000
Glucose	700.000
D-Fructose	400.000
Fumaric acid	55.000
L-Malic acid	670.000
Lactalbumin hydrolysate	3330.000
Succinic acid	60.000
Sucrose	26680.000
Yeast extract	3330.000

Methodology

1. Suspend 52.4gms 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 precipitaion.
4. Make up the final volume to 1000ml.
5. Adjust the osmolality as desired. For Lepidopterans cell line, osmolality of 340 - 360mOsm/KgH_2O is recommended. The osmolality can be increased by 10mOsm/KgH_2O 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/KgH2O by adding 27.8ml of water to per litre of medium.
6. Sterilize the medium using a membrane filter with porosity of 0.22 microns or less.
7. Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into sterile containers.
8. 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)
Potassium chloride (TC1010)
Foetal bovine serum (BA3112/ BA12432)

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Quality control

Appearance
Off-white to Creamish white, homogenous powder.
Solubility
Solution with some particles at 52.4gms/L.
pH without Sodium Bicarbonate
4.00 -4.60
pH with Sodium Bicarbonate
4.30 -4.90
Osmolality without Sodium Bicarbonate
335.00 -375.00
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 15EU/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. 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. 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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