

Technical Information

Waymouth Medium MB 752/1

With L-Glutamine

Without Sodium bicarbonate

Product Code:AT1091

Application: - Waymouth Medium MB 752/1 was developed in 1959 for the cultivation of mouse L cells, NCTC clone929. Waymouth's medium is a comparatively simple medium with lesser ingredients than its other synthetic counter parts. The medium comprises of amino acids, vitamins, the purine base hypoxanthine, salts, and glucose. This medium can be used for culturing many fastidious cell lines and also whole organ culture.

AT1091 is Waymouth Medium MB 752/1 with L- glutamine. 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 dihydrate	120.000
Disodium hydrogen phosphate anhydrous	300.000
Magnesium chloride anhydrous	112.560
Magnesium sulphate anhydrous	97.720
Potassium chloride	150.000
Potassium dihydrogen phosphate	80.000
Sodium chloride	6000.00.

AMINO ACIDS

Glycine	50.000
L-Arginine hydrochloride	75.000
L-Aspartic acid	60.000
L-Cysteine dihydrochloride	90.000
L-Cystine dihydrochloride	19.550
L-Glutamic acid	150.000
L-Glutamine	350.000
L-Histidine hydrochloride monohydrate	150.000
L-Isoleucine	25.000
L-Leucine	50.000
L-Lysine hydrochloride	240.000
L-Methionine	50.000
L-Phenylalanine	50.000
L-Proline	50.000
L-Threonine	75.000
L-Tryptophan	40.000
L-Tyrosine Disodium Salt	57.660
L-Valine	65.000

VITAMINS

Ascorbic acid	17.500
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Biotin	0.020
Choline chloride	250.000
D-Ca-Pantothenate	1.000
Folic acid	0.400
Niacinamide	1.000
Pyridoxine hydrochloride	1.000
Riboflavin	1.000
Thiamine hydrochloride	10.000
Vitamin B12	0.200
α-Inositol	1.000
OTHERS	
D-Glucose	5000.00
Glutathione reduced	15.000
Hypoxanthine sodium salt	29.000
Phenol red sodium salt	11.000

Methodology

1. Suspend 13.8gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 2.24gms of sodium bicarbonate powder (TC1230) or 29.87ml of 7.5% sodium bicarbonate solution (TCL1013) for 1litre of medium and stir until dissolved.
3. Adjust the pH to 0.2 - 0.3 pH units below the desired pH using 1N HCl or 1N NaOH since the pH tends to rise during filtration
4. Make up the final volume to 1000ml with tissue culture grade water
5. Sterilize the medium immediately by filtering through a sterile membrane filter with a porosity of 0.22 micron or less, using positive pressure rather than vacuum to minimize the loss of carbon dioxide.
6. Aseptically add sterile supplements as required and dispense the desired amount of sterile medium into 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)
 Sodium bicarbonate (TC1230)
 Sodium bicarbonate solution, 7.5% (TCL1013)
 1N Hydrochloric acid (TCL1003)
 1N Sodium hydroxide (TCL1002)
 Foetal bovine serum (BA3112/BA30432)

Quality Control

Appearance

Off-white to Creamish white, homogenous powder .

Solubility

Clear solution at 13.8 gms/L

pH without Sodium Bicarbonate

5.20-5.80

pH with Sodium Bicarbonate

7.10-7.70

Osmolality without Sodium Bicarbonate

260.00-300.00

Osmolality with Sodium Bicarbonate

305.00-345.00

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 sub cultures.

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. 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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