CDH

Product Specification

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Technical Information

RPMI 1640

With 25mM HEPES buffer

Without L-Glutamine, Phenol red, and Sodium bicarbonate

Product Code: AT1113A

Application:- Roswell Park Memorial Institute (RPMI) media are a series of media developed by Moore et al for the culture of human normal and neoplastic cells in vitro. RPMI1640 is the most commonly used medium in the series A modification of McCoy's 5A medium, the medium was specifically designed to support the growth of human lymphoblastoid cells in suspension culture. Presently the medium is extensively used for a wide range of anchorage dependant cell lines. The medium needs to be supplemented with 5-20% fetal bovine serum. The medium is also known to support growth of cells in the absence of serum.

AT1113A is RPMI 1640 with 25mM HEPES buffer. It does not contain L-glutamine, phenol red and sodium bicarbonate. HEPES, a zwitterionic buffer having a pKa of 7.3 at 37ºC prevents the initial rise in pH that tends to occur at the initiation of a culture and increases the buffering capacity of the medium. 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 nitrate tetrahydrate	100.00
Magnesium sulphate anhydrous	48.840
Potassium chloride	400.000
Sodium chloride	6000.000
Sodium phosphate dibasic anhydrous	800.00
AMINO ACIDS	
Glycine	10.000
L-Arginine hydrochloride	241.870
L-Asparagine anhydrous	50.000
L-Aspartic acid	20.000
L-Cysteine dihydrochloride	65.200
L-Glutamic acid	20.000
L-Histidine hydrochloride monohydrate	18.520
L-Hydroxy-Proline	20.000
L-Isoleucine	50.000
L-Leucine	50.000
L-Lysine hydrochloride	40.000
L-Methionine	15.000
L-Phenylalanine	15.000
L-Proline	20.000
L-Serine	30.000
L-Threonine	20.000
L-Tryptophan	5.000
L-Tyrosine Disodium Salt	28.830
L-Valine	20.000

VITAMINS	
Choline chloride	3.000
D-Biotin	0.200
D-Calcium pantothenate	0.250
Folic acid	1.000
Niacinamide	1.000
Pyridoxine hydrochloride	1.000
Riboflavin	0.200
Thiamine hydrochloride	1.000
Vitamin B12	0.005
i-Inositol	35.000
p-Aminobenzoic acid (PABA)	1.000
OTHERS	
D-Glucose	2000.000
Glutathione reduced	1.000
HEPES Buffer	5958.00

Methodology

1. Suspend 16.1 gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 2.0gms of sodium bicarbonate powder (TC1230) or 26.7ml of 7.5% sodium bicarbonate solution (TCL1013) and10.3ml of 200mM L-glutamine solution (TCL1012) for 1 litreof 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)
L-Glutamine powder (TC1243)
L-Glutamine solution 200mM (TCL1012)
Foetal bovine serum (BA3112/BA30432)

Quality Control

Appearance
Off-white to Creamish white, homogenous powder .

Solubility
Clear solution at 16.1gms/L

pH without Sodium Bicarbonate
6.40--7.00

pH with Sodium Bicarbonate
6.70-7.30

Osmolality without Sodium Bicarbonate
280.00-320.00

Osmolality with Sodium Bicarbonate
320.00-360.00

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 2-8ºC. 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.
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