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Technical Datasheet

McCoy's 5A Medium

(For suspension culture)
With L-Glutamine
Without Calcium chloride and Sodium bicarbonate

Product Code: AT071

Product Description:

McCoy's 5A medium was developed at Roswell Park Memorial Institute in Buffalo, New York. The first medium was developed in 1955 as the result of studies on the nutritional requirements of the Walker 256 carcinoma. The original formulation was based on the amino acids in concentrations similar to those in Eagle's medium as well as the water soluble vitamins of Medium 199. Modifications to the original formulation resulted in the final version which was published in 1960. The final formulation also incorporates modifications done by Iwakata and Grace and contains increased amounts of folic acid, vitamin B12 and peptone. This medium is also known to support growth of primary cultures derived from a variety of tissues.

AT071 is McCoy's 5A medium with L-Glutamine. It is modified for use with suspension cells, hence does not contain calcium chloride. Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

Composition:

Ingredients	mg/L
INORGANIC SALTS	
Magnesium sulphate anhydrous	97.720
Potassium chloride	400.000
Sodium chloride	6460.000
Sodium phosphate monobasic anhydrous	1220.000
AMINO ACIDS	
Glycine	7.510
L-Alanine	13.360
L-Arginine hydrochloride	42.140
L-Asparagine monohydrate	45.030
L-Aspartic acid	19.970
L-Cysteine hydrochloride	31.500
L-Glutamic acid	22.070
L-Glutamine	219.200
L-Histidine hydrochloride monohydrate	20.960
L-Hydroxyproline	19.670
L-Isoleucine	39.360
L-Leucine	39.360
L-Lysine hydrochloride	36.540
L-Methionine	14.920
L-Phenylalanine	16.520
L-Proline	17.270
L-Serine	26.280
L-Threonine	17.870
L-Tryptophan	3.060
L-Tyrosine disodium salt dihydrate	26.100
L-Valine	17.570
VITAMINS	
Ascorbic acid sodium salt	0.6330
Biotin	0.200
Choline chloride	5.000
D-Ca-Pantothenate	0.200
Folic acid	10.000
Niacin	0.500
Niacinamide	0.500
Pyridoxal hydrochloride	0.500
Pyridoxine hydrochloride	0.500
Riboflavin	0.200
Thiamine hydrochloride	0.200
Vitamin B12	2.000
i-Inositol	36.000
p-Amino benzoic acid (PABA)	1.000
OTHERS	
D-Glucose	3000.000
Glutathione reduced	0.500
Peptic digest of animal tissue	600.000
Phenol red sodium salt	11.000

Directions:

1. Suspend 12.5gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 2.2gms of sodium bicarbonate powder (TC230) or 29.3ml of 7.5% sodium bicarbonate solution (TCL013) for 1 litre 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 (TCL010)
Sodium bicarbonate (TC230)
Sodium bicarbonate solution, 7.5% (TCL013)
1N Hydrochloric acid (TCL003)
1N Sodium hydroxide (TCL002)
Fetal bovine serum(RM1112/RM10432)

Quality Control:

Appearance
White to light pink, homogenous powder

Solubility
Clear solution at 12.5 gms/L.

pH without Sodium Bicarbonate
4.50 - 5.10

pH with Sodium Bicarbonate
6.50 - 7.10

Osmolality without Sodium Bicarbonate(mOSm/Kg H_2O)
250.00 - 290.00

Osmolality with Sodium Bicarbonate(mOsm/Kg H_2O)
290.00 - 330.00

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.

Endotoxin Content
NMT 1EU/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. 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.