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Technical Datasheet

Dulbecco's Modified Eagle Medium (DMEM), High glucose

With L-Glutamine, 4.5gm Glucose per litre, Sodium pyruvate and 25mM HEPES buffer Without Sodium bicarbonate

Product Code: AT151

Product Description:

Dulbecco's Modified Eagle Medium is one of the most widely used modification of Eagles medium. DMEM is a modification of Basal Medium Eagle (BME) that contains four fold concentration of amino acids and vitamins. Additionally, the formulation also includes glycine, serine and ferric nitrate. The original formulation contains 1000mgs glucose per litre and was originally used to culture embryonic mouse cells.

DMEM high glucose is a further modification of original DMEM and contains 4500mgs glucose per litre. The additional glucose has proved to be useful in cultivating various other cell lines including primary cultures of mouse and chicken cells as well as various normal and transformed cell lines.

AT151 is DMEM with 4.5gms glucose per litre, L-glutamine, sodium pyruvate and 25mM HEPES Buffer. 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/L
INORGANIC SALTS	
Calcium chloride dihydrate	265.000
Ferric nitrate nonahydrate	0.100
Magnesium sulphate anhydrous	97.720
Potassium chloride	400.000
Sodium chloride	4400.000
Sodium dihydrogen phosphate anhydrous	109.000
AMINO ACIDS	
Glycine	30.000
L-Arginine hydrochloride	84.000
L-Cystine dihydrochloride	62.570
L-Glutamine	584.000
L-Histidine hydrochloride monohydrate	42.000
L-Isoleucine	105.000
L-Leucine	105.000
L-Lysine hydrochloride	146.000
L-Methionine	30.000
L-Phenylalanine	66.000
L-Serine	42.000
L-Threonine	95.000
L-Tryptophan	16.000
L-Tyrosine disodium salt dihydrate	103.790
L-Valine	94.000
VITAMINS	
Choline chloride	4.000
D-Ca-Pantothenate	4.000
Folic acid	4.000
Nicotinamide	4.000
Pyridoxal 5 phosphate	4.000
Riboflavin	0.400
Thiamine hydrochloride	4.000
i-Inositol	7.200
OTHERS	
D-Glucose	4500.000
HEPES Buffer	5958.000
Phenol red sodium salt	15.900
Sodium pyruvate	110.000

Directions:

1. Suspend 17.5gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 3.7gms of Sodium bicarbonate powder (TC230) or 49.3ml 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 17.5 gms/L.

pH without Sodium Bicarbonate
5.90 - 6.50

pH with Sodium Bicarbonate
6.70 - 7.30

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

Osmolality with Sodium Bicarbonate (mOsm/Kg H_2O)
330.00 - 370.00

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. 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.