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Technical Datasheet

SFRE Medium 199-2

With Earle's Salts, L-Glutamine, Galactose and Glucose
Without Sodium bicarbonate and Insulin

Product Code: AT090

Product Description :

SFRE Medium 199 is modification of medium 199 developed for growth and maintenance of primary baboon kidney (Bak) cells. Both the media were formulated by supplementing medium M199 with insulin, sodium pyruvate, zinc sulfate, and increasing arginine-HCl, cysteine, cystine, L-glutamine, L-glutamic acid, glycine, histidine, tyrosine, and glucose to maximally active nontoxic concentrations. SFRE 199-2 is additionally supplemented with galactose to avoid excessive accumulation of lactic acid and to maintain pH in the physiological range for prolonged maintenance of the cells.

AT090 is SFRE Medium 199-2 with Earle's salts, L-glutamine, galactose and glucose. It does not contain insulin, hence has to be added separately prior to use. 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	0.720
Magnesium sulphate anhydrous	97.720
Potassium chloride	400.000
Sodium acetate	50.000
Sodium chloride	6800.000
Sodium dihydrogen phosphate anhydrous	122.000
Zinc sulphate heptahydrate	0.100
AMINO ACIDS	
Glycine	100.000
Hydroxy-L-Proline	10.000
L-Alanine	25.000
L-Arginine hydrochloride	150.000
L-Aspartic acid	30.000
L-Cysteine hydrochloride monohydrate	4.000
L-Cystine dihydrochloride	43.800
L-Glutamic acid	75.000
L-Glutamine	300.000
L-Histidine hydrochloride monohydrate	40.000
L-Isoleucine	20.000
L-Leucine	60.000
L-Lysine hydrochloride	70.000
L-Methionine	15.000
L-Phenylalanine	25.000
L-Proline	40.000
L-Serine	25.000
L-Threonine	30.000
L-Tryptophan	10.000
L-Tyrosine disodium salt dihydrate	116.000
L-Valine	25.000
VITAMINS	
Calciferol	0.100
Choline chloride	0.500
D-Biotin	0.010
D-Ca-Pantothenate	0.010
DL-Tocopherol phosphate disodium salt	0.010
Folic acid	0.010
L-Ascorbic acid	0.050
Menadione sodium bisulphite	0.016
Niacin	0.025
Niacinamide	0.025
Pyridoxal hydrochloride	0.025
Pyridoxine hydrochloride	0.025
Retinol Acetate	0.140
Riboflavin	0.010
Thiamine hydrochloride	0.010
i-Inositol	0.050
p-Amino benzoic acid (PABA)	0.050
OTHERS	
Adenine sulphate	10.000
Adenosine monophosphate	1.000
Adenosine triphosphate	0.200
Cholesterol	0.200
D(+) Galactose anhydrous	1000.000
D-Glucose	2000.000
Deoxyribose	0.500

Glutathione reduced	0.050
Guanine hydrochloride	0.300
Hypoxanthine	0.354
Phenol red sodium salt	10.000
Polysorbate 80	4.900
Ribose	0.500
Sodium pyruvate	150.000
Thymine	0.300
Uracil	0.300
Xanthine	0.344

Directions :

1. Suspend 12.1gms in 900 ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 2.20gms 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)
Foetal bovine serum (RM1112/RM10432)

Quality Control:

Appearance
White to light pink, homogenous powder

Solubility
Clear solution at 12.1 gms/L.

pH without Sodium Bicarbonate
4.70 -5.30

pH with Sodium Bicarbonate
7.20 -7.80

Osmolality without Sodium Bicarbonate(mOsm/Kg H_2O)
260.00 -300.00

Osmolality with Sodium Bicarbonate(mOsm/Kg H_2O)
300.00 -340.00

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.