

### Technical Information

#### Medium 199

With Earle's salts Without L-Glutamine and Sodium bicarbonate

**Product Code : AT1014A**

**Application:-** Medium 199 was the first nutritionally defined medium developed by Morgan, Morton, and Parker in 1950. This complex medium was formulated specifically for nutritional studies on primary chick embryo fibroblasts in the absence of any additives. It was observed that explanted tissue could survive in Medium 199 without serum but long term cultivation of cells required supplementation of the medium with serum. Medium 199 is formulated with either Hank's salts or Earle's salts. The medium when supplemented with serum can be used for growth of a wide variety of cells. Medium 199 is presently used for the maintenance of non-transformed cells, vaccine and virus production and primary explants of epithelial cells. AT1014A is Medium 199 with Earle's salts. It does not contain 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                           | Gms / Litre |
|---------------------------------------|-------------|
| <b>INORGANIC SALTS</b>                |             |
| Calcium chloride dihydrate            | 265.000     |
| Ferric nitrate nonahydrate            | 0.720       |
| Magnesium sulphate anhydrous          | 97.720      |
| Potassium chloride                    | 400.000     |
| Sodium acetate anhydrous              | 50.000      |
| Sodium chloride                       | 6800.000    |
| Sodium phosphate, monobasic           | 122.000     |
| <b>AMINO ACIDS</b>                    |             |
| Glycine                               | 50.000      |
| L-Alanine                             | 25.000      |
| L-Arginine hydrochloride              | 70.00       |
| L-Aspartic acid                       | 30.000      |
| L-Cysteine hydrochloride monohydrate  | 0.100       |
| L-Cystine dihydrochloride             | 26.000      |
| L-Glutamic Acid                       | 67.000      |
| L-Histidine hydrochloride monohydrate | 22.000      |
| L-Hydroxyproline                      | 10.000      |
| 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              | 57.660      |
| L-Valine                              | 25.000      |

### VITAMINS

|                                       |       |
|---------------------------------------|-------|
| Ascorbic acid                         | 0.050 |
| 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 |
| Menadione                             | 0.010 |
| Nicotinamide                          | 0.025 |
| Nicotinic acid                        | 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 | 0.200    |
| Adenosine triphosphate  | 1.000    |
| Cholesterol             | 0.200    |
| Deoxyribose             | 0.500    |
| Glucose                 | 1000.000 |
| Glutathione reduced     | 0.050    |
| Guanine hydrochloride   | 0.300    |
| Hypoxanthine            | 0.354    |
| Phenol red sodium salt  | 15.000   |
| Polysorbate 80          | 4.900    |
| Ribose                  | 0.500    |
| Thymine                 | 0.300    |
| Uracil                  | 0.300    |
| Xanthine                | 0.344    |

## Methodology

1. Suspend 9.4gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water .
2. Add 0.1gms of L-glutamine powder (TC1230) or 3.42ml of 200mM L-glutamine (TCL1012) 2.2gms of sodium bicarbonate powder (TC1243) or 29.3 ml of 7.5% sodium bicarbonate solution (TCL1013) 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 (TCL1010)  
Sodium bicarbonate (TC1230)  
Sodium bicarbonate solution, 7.5% (TCL1013)  
L-Glutamine powder (TC1243)  
L-Glutamine solution 200mM (TCL1012)  
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 9.4gms/L

#### pH without Sodium Bicarbonate

4.40-5.00

#### pH with Sodium Bicarbonate

7.20-7.80

#### Osmolality without Sodium Bicarbonate

230.00 -270.00

#### Osmolality with Sodium Bicarbonate

265.00-305.00

#### 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 2-8°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.
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