AME's Medium
With L-Glutamine
Without Phenol red and Sodium bicarbonate

Product Code: AT121

Product Description:
AME's medium was specially formulated to maintain rabbit retina in vitro. Rabbit retina is a good model for studying relationships between function and metabolism in organized mammalian central nervous tissue. Rabbit retina offers several advantages as it is easily accessible and strong enough to remain intact during manipulations. AME's medium is formulated to closely resemble the composition of the cerebrospinal fluid that bathes the retina in vivo. Morphologic, metabolic, and electrophysiologic measurements obtained on the in vitro retinas showed that they remained in a nearly physiological state for at least 8 h, and even after 2 days in vitro they still exhibited a high level of metabolic activity and electrical responsiveness to light. AME's medium therefore remains the medium of choice for maintaining central nervous tissue in vitro.

AT121 is AME's Medium with L-glutamine. It does not contain Phenol red and Sodium bicarbonate. 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   169,000
Magnesium sulphate anhydrous 149,270
Potassium chloride           231,000
Potassium dihydrogen phosphate 68,000
Sodium chloride              7010,000

AMINO ACIDS
Glycine                     0.450
L-Alanine                    2.400
L-Arginine hydrochloride    4.210
L-Asparagine anhydrous      0.84
L-Aspartic acid             0.12
L-Cystine dihydrochloride   0.065
L-Glutamic acid Sodium salt 1.183
L-Glutamine                 73,000
L-Histidine hydrochloride   2,513
L-Isoleucine                0.580
L-Leucine                   1.440
L-Lysine hydrochloride      3.648
L-Methionine                0.390
L-Phenylalanine             1.320
L-Proline                   0.070
L-Serine                    2.520
L-Threonine                 3.330
L-Tryptophan                0.490
L-Tyrosine Disodium salt    1.820
L-Valine                    1.760
Taurine                     0.750

VITAMINS
Ascorbic acid Sodium salt   17.960
Choline chloride            0.700
Cytidine                    0.730
D-Biotin                    0.100
D-Ca-Pantothenic acid (Hemicalcium) 0.100
Folic acid                  0.100
Nicinamide                  0.100
Pyridoxal hydrochloride     0.100
Riboflavin                  0.010
Thiamine hydrochloride      0.100
myo-Inositol                27.200

OTHERS
D-Glucose                   1081.000
Hypoxanthine                0.820
Sodium pyruvate             13.330
Thymidine                   0.240
Uridine                     0.730

Directions:
1. Suspend 8.9gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 1.9gms of sodium bicarbonate powder (TC230) or 25.3ml 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**
Off-white to Creamish white, homogenous powder.

**Solubility**
Clear solution at 8.9 gms/L.

**pH without Sodium Bicarbonate**
4.30 - 4.90

**pH with Sodium Bicarbonate**
7.40 - 8.00

**Osmolality without Sodium Bicarbonate**
230.00 - 270.00

**Osmolality with Sodium Bicarbonate**
260.00 - 300.00

**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. 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.