Chick Embryo Fibroblast Medium
With L-Glutamine
Without Sodium bicarbonate

Product Code: AT168

Product Description:
Chicken embryo is a widely used medium for the initial isolation and propagation of viruses and the production of vaccines. Chick Embryo Fibroblast Medium is a proprietary formulation specially developed to culture chicken embryo fibroblast cells.

AT168 is Chick Embryo Fibroblast Medium with L-glutamine. Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

Composition:
This medium is a proprietary formulation containing inorganic salts, essential and non-essential amino acids, vitamins, growth factors, trace elements and glucose. It does not contain sodium bicarbonate.

Directions:
1. Suspend 11.33gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 2.75gms of sodium bicarbonate powder (TC230) or 36.67ml 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 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)

Quality Control:

Appearance
Off-white to Creamish white, homogenous powder.

Solubility
Clear solution at 11.3 gms/L.

pH without Sodium Bicarbonate
5.30 - 5.90

pH with Sodium Bicarbonate
7.40 - 8.00

Osmolality without Sodium Bicarbonate
200.00 - 240.00

Osmolality with Sodium Bicarbonate
250.00 - 290.00

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.