

Click's Medium (EHAA) Eagle Hanks' Amino Acids

Without L-Glutamine, Mercaptoethanol and Sodium bicarbonate

Product Code:AT122A

Product Description :

Click's medium was developed by Robert Click to study the in vitro immune response of mouse spleen cells. The addition of mercaptoethanol to the medium permits successful immunization of spleen cells with heterologous RBC cultured under stationary conditions without daily feeding.

AT122A is Click's Medium (EHAA) Eagle Hanks' Amino Acids without L-glutamine and mercaptoethanol. 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 Disodium hydrogen phosphate Magnesium chloride anhydrous Potassium chloride	245.530
Potassium dihydrogen phosphate	47.900
Sodium chloride	164.900
	400.000
	60.000
	8000.000
AMINO ACIDS	
Glycine	30.000
L-Alanine	35.600
L-Arginine hydrochloride	317.500
L-Asparagine anhydrous	52.800
L-Aspartic acid	53.200
L-Cystine dihydrochloride	78.200
L-Glutamic acid	58.800
L-Histidine hydrochloride monohydrate	104.700
L-Isoleucine	130.000
L-Leucine	130.000
L-Lysine hydrochloride	181.200
L-Methionine	37.500
L-Phenylalanine	80.000
L-Proline	46.000
L-Serine	42.000

L-Threonine	120.000
L-Tryptophan	25.000
L-Tyrosine disodium salt dihydrate	118.900
L-Valine	115.000
VITAMINS	
Calcium pantothenate Choline chloride	2.000
Folic acid	2.000
Niacinamide	2.000
Pyridoxal hydrochloride	2.000
Riboflavin	0.200
Thiamine hydrochloride	2.000
myo-Inositol	4.000
OTHERS	
Adenosine	25.000
Cytosine	25.000
Glucose	1000.000
Guanosine	25.000
Magnesium sulphate anhydrous	97.720
Phenol red Sodium Salt	11.000
Sodium pyruvate	275.000
Uridine	25.000

Directions:

1. Suspend 12.2gms in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 1.35gms of sodium bicarbonate powder (TC230) or 18.0ml of 7.5% sodium bicarbonate solution (TCL013) and 0.584gms of L-glutamine powder (TC243) or 20ml of 200mM L-glutamine solution (TCL012) 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)
L-Glutamine powder (TC243)
L-Glutamine solution, 200mM (TCL012)
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 12.2 gms/L.

pH without Sodium Bicarbonate

4.80 -5.40

pH with Sodium Bicarbonate

7.10 -7.70

Osmolality without Sodium Bicarbonate(mOsm/Kg H₂O)

280.00 -320.00

Osmolality with Sodium Bicarbonate(mOsm/Kg H₂O)

320.00 -360.00

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.

Disclaimer :

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