

HybridoXL™ Medium

With L-Glutamine, Insulin and HEPES buffer
Without Sodium bicarbonate

Product Code: AT191

Product Description :

HybridoXL™ Medium is a medium specially developed for propagation of hybridomas and other fastidious cell lines. It is a modified mixture of Dulbecco's Modified Eagle Medium and NCTC 135. On supplementation with 10% fetal bovine serum and insulin, it supports rapid growth of many hybridoma cell lines.

AT191 is HybridoXL™ medium with L-glutamine and HEPES buffer. HEPES, a zwitterionic buffer having a pKa of 7.3 at 37°C prevents the initial rise in pH that tends to occur at the initiation of a culture and increases the buffering capacity of the medium. It does not contain insulin and sodium bicarbonate. This medium is supplied in two parts. Part A contains chemically defined media powder and Part B contains recombinant human insulin powder. Users are advised to review the literature for recommendations regarding medium supplementation and physiological growth requirements specific for different cell lines.

Composition (Part A) :

Ingredients	mg/L
INORGANIC SALTS	
Calcium chloride anhydrous	191.400
Ferric nitrate nonahydrate	0.087
Magnesium sulfate anhydrous	93.470
Potassium chloride	382.800
Sodium acetate trihydrate	4.350
Sodium chloride	6159.600
Sodium glucuronate monohydrate	0.1566
Sodium phosphate monobasic monohydrate	120.930
AMINO ACIDS	
Glycine	33.800
L-Alanine	10.480
L-Arginine hydrochloride	75.790
L-Asparagine monohydrate	12.290
L-Aspartic acid	12.430
L-Cystine dihydrochloride	55.630
L-Glutamic acid	13.510

L-Glutamine	773.930
L-Histidine hydrochloride monohydrate	38.860
L-Hydroxyproline	0.356
L-Isoleucine	92.920
L-Leucine	93.130
L-Lysine hydrochloride	130.360
L-Methionine	26.490
L-Ornithine hydrochloride	0.819
L-Phenylalanine	58.860
L-Proline	10.540
L-Serine	46.610
L-Threonine	84.300
L-Tryptophan	15.440
L-Tyrosine disodium salt	92.360
L-Valine	83.960
Taurine	0.364
VITAMINS	
Ascorbic acid	4.350
Biotin	0.00218
Choline chloride	3.588
D-Ca-Pantothenate	3.482
Folic acid	3.482
Menadione	0.0218
Niacinamide	3.485
Nicotinic acid	0.00548
Pyridoxal hydrochloride	3.485
Pyridoxine hydrochloride	0.00548
Riboflavin	0.350
Thiamine hydrochloride	0.00218
Vitamin A Alcohol	0.0218
Vitamin B-12	0.870
Vitamin D-2	0.0218
alpha-Tocopherol phosphate	0.00218
i-Inositol	6.274
p-Aminobenzoic acid (PABA)	0.0109
OTHERS	
2-Deoxyadenosine	0.870
2-Deoxycytidine hydrochloride	0.870
2-Deoxyguanosine	0.870
5-Methylcytosine	0.0087

Coccarboxylase	0.087
Coenzyme A	0.217
D-Glucose	957.000
D-Glucuronolactone	0.157
FAD disodium dihydrate	0.087
Glutathione reduced	0.870
HEPES buffer	2073.210
NAD DPN	0.609
NADP TPN	0.087
Oxaloacetic acid	114.840
Phenol red	14.790
Sodium pyruvate	57.420
Thymidine	0.870
Tween 80	1.087
Uridine-5-triphosphate	0.087

Composition (Part B) :

Recombinant Human Insulin Powder	7.000
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Directions :

1. Suspend 12.0gms of AT191-Part A in 900ml tissue culture grade water with constant, gentle stirring until the powder is completely dissolved. Do not heat the water.
2. Add 1.5gms of sodium bicarbonate powder (TC230) or 20ml of 7.5% sodium bicarbonate solution (TCL013) and 7mg of Recombinant Human Insulin AT191-Part B 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 powder (TC230)
Sodium bicarbonate solution, 7.5% (TCL013)
1N Hydrochloric acid (TCL003)
1N Sodium hydroxide (TCL002)
Fetal Bovine Serum, Hybridoma tested (RM10906)

Quality Control:

Appearance

Off-white to Creamish white, homogenous powder

Solubility

Clear solution at 12.0gms/L

pH without Sodium Bicarbonate

5.00 -5.60

pH with Sodium Bicarbonate

7.30 -7.90

Osmolality without Sodium Bicarbonate

235.00 -275.00

Osmolality with Sodium Bicarbonate

290.00 -330.00

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.

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.

Revision : 0 / 2015



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