BSK-H Medium Base

M1668

BSK-H Medium Base is a chemically defined medium for the cultivation of *Borrelia burgdorferi* .

Composition**

Ingredients	Gms / Litre
Part A	-
Bovine Serum Albumin	50.000
Part B	-
L-Cysteine.HCl.H2O	0.260
L-Cystine	0.020
L-Glutamic acid	0.075
Glycine	0.050
L-Histidine HCl. H2O	0.020
Trans-4-Hydroxy-L-Proline	0.010
L-Isoleucine	0.020
L-Leucine	0.060
L-Lysine.HCl	0.070
L-Methionine	0.015
L-Phenylalanine	0.025
L-Proline	0.040
L-Serine	0.025
L-Threonine	0.030
L-Tryptophan	0.010
L-Tyrosine	0.040
L-Valine	0.025
N-Acetyl-D-Glucosamine	0.400
L-Ascorbic acid	0.050
PABA	0.00005
D-Biotin	0.00001
Choline chloride	0.0005
Citric acid.3Na.2H2O	0.700
Coenzyme A	0.0025
Cocarboxylase	0.001
2'-Deoxyadenosine	0.010
2'-Deoxyguanosine	0.010
2'-Deoxycytidine.HCl	0.0116
Flavin Adenine Dinucleotide.2Na	0.000106
Folic acid	0.00001
myo-Inositol	0.00005
5-Methyldeoxycytidine	0.0001
Nicotinamide adenine dinucleotide	0.007
Nicotinamide adeninedinucleotide phosphate	0.001
Niacinamide	0.000025
Nicotinic acid	0.000025
D-Pantothenic Acid-Hemicalcium	0.00001
Pyridoxal.HCl	0.000025
Pyridoxine.HCl	0.000025
Pyruvic Acid.Na	0.800
Riboflavin	0.00001
Thiamine.HCl	0.00001
Thymidine	0.010
Uridine-5-Triphosphate.Na	0.001
Calcium Chloride (anhydrous)	0.200

Magnesium Sulphate (anhydrous)	0.09769
Potassium Chloride	0.400
Sodium Acetate (anhydrous)	0.050
Sodium Bicarbonate	2.200
Sodium Chloride	6.800
Sodium Phosphate monobasic (anhydrous)	0.122
D-Glucose	6.000
Phenol Red. Na	0.02124
Glutathione	0.010
D-Glucuronic acid.Na	0.00388
Cholesterol	0.0002
Tween 80	0.005
HEPES	6.000
Peptone, special	5.000
Yeast Extract	2.000
L-Alanine	0.025
L-Arginine	0.05787
L-Asparatic acid	0.030
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25 grams of part A in 450 ml distilled water. Mix well to dissolve the medium completely. To this add 15.9 grams of Part B. Add distilled water to make the final volume to 500 ml. Mix thoroughly to get a clear solution. Sterilize by filtration. DO NOT AUTOCLAVE. Aseptically add entire contents of one vial of Antibiotic Mixture for Borrelia (FD179) and 30 ml of Rabbit Serum (FD180). Mix well and dispense as desired.

Principle And Interpretation

BSK-H Medium is a modification of BSK-II medium developed by Pollack et al. (1) for the cultivation of *Borrelia burgdorferi* . *Borrelia* species are relatively slow-growing and their nutritional requirements appear to be complex. The study of *Borrelia* was greatly facilitated by the development of a culture medium by Kelly (2) that supported the growth of Spirochaetes. Stoenner enriched the basic formulation of Kelly with the addition of yeast extract tissue culture medium (3). Subsequent modifications by Barbour (4) resulted in BSK (Barbour-Stoenner-Kelly) medium, which facilitated isolation of *Borrelia* form a variety of tissue.

This medium is complex mixture of different amino acids, vitamins and growth factors which are required for the growth of *Borrelia* and *Spirochaete* , it is enriched with bovine albumin and rabbit serum. Peptone, special serves as nitrogen source while glucose as energy source. Cholesterol incorporated in the medium acts as source of lipid. The success of in vitro culture of *Borrelia* is usually dependent on the quality of the animal serum or albumin used in media preparation (5). HEPES provides buffering capacity to the medium while different salts of Magnesium, sodium, calcium and potassium maintain the ionic balance in the medium.

Quality Control

Appearance

Part A : Off-white to pale yellow Free flowing flakes Part B : Light yellow to pink homogeneous free flowing powder

Colour and Clarity of prepared medium

Light orange coloured, clear transparent liquid forms in tubes.

Reaction

Reaction of 8.2% w/v aqueous solution (Part A and Part B] at 25°C. pH : 7.6±0.2

pH

7.40-7.80

Microbial Load

Maximum Limit

Cultural Response

M1668: Cultural characteristics observed after 1-4 weeks incubation at 30-35°C after addition of Antibiotic Mixture for Borrelia (FD179) and of Rabbit Serum (FD180).

Organism	Growth
Cultural Response	
Escherichia coli ATCC 25922	Inhibited
Borrelia burgdorferi ATCC 35210	Fair to good
Staphylococcus aureus ATCC 25923	Inhibited

Storage and Shelf Life

Store at 2 - 8°C in tightly closed container. Use before expiry date on the label

Reference

1.Pollack R.J. et al, 1993, J. Clin. Microbiol., 31:1251-5.
2.Kelly, R., 1971, Science, 173:443-444.
3.Stoenner, H.G. et al, 1982, J. Exp. Med., 156:1297-1311.
4.Barbour, A.G., 1984, J. Biol. Med. , 57:521-525.
5.Calister, S.M, et al., 1990, J. Clin. Microbiol., 28:363-365.

Revision : 1 / 2011