BRAIN HEART INFUSION AGAR

- For in vitro use only -

Plated Media
PB50 – Brain Heart Infusion Agar (BHIA)
PB55DP – BHIA w 10% Sheep Blood (Double Pour)
PB54DP – BHIA w 10% SB & CC (Double Pour)
PB56 – BHIA w 5% Sheep Blood
PB56DP – BHIA w 5% Sheep Blood (Double Pour)
PB58DP – BHIA w 5% SB & CC (Double Pour)

Tubed Media
TB50-05 – BHIA Slant
TB50-18 – BHIA Pour Plate
TB51 – BHIA w 10% Sheep Blood Slant
TB52 – BHIA w 5% Sheep Blood Slant

Brain Heart Infusion Agar is a general purpose plating medium used for the isolation, cultivation, and maintenance of a variety of fastidious and non-fastidious microorganisms.

In 1919, Rosenow devised an excellent medium for culturing streptococci by using a dextrose broth supplemented with brain tissue. Rosenow's formulation was later modified by Hayden whom found the addition of crushed marble resulted in favorable growth of dental pathogens. Our current formulation contains infusion from calf brain in place of brain tissue and disodium phosphate has replaced calcium carbonate.

BHI Agar is a highly nutritious base that meets the growth requirements of many types of microorganisms including bacteria, yeasts, and molds. BHI Agar supplemented with (5 to 10%) defibrinated sheep blood is used extensively for the recovery of dimorphic fungi such as *Histoplasma capsulatum* and other pathogenic fungi such as *Coccidioides immitis*. A more selective formulation containing chloramphenicol and cycloheximide is also available that will allow the recovery of pathogenic fungi while inhibiting a wide range of bacteria and saprophytic fungi. McDonough et al. demonstrated that the temperature of incubation may alter the sensitivity of some pathogenic fungi to antibiotics; it is therefore recommended that both an antimicrobic-containing medium and non-selective medium be used on primary isolates at both 25°C and 35°C.

The double pour media is poured extra thick to maintain the moisture level of the medium where prolonged incubation of the media is required.

Formulation per Litre of Medium

PB50 & TB50 Brain Heart Infusion Agar

Infusion from Calf Brain	12.5 g
Infusion from Beef Heart	5.0 g
Pancreatic Digest of Casein	10.0 g
Sodium Chloride	5.0 g
Glucose	2.0 g
Disodium Phosphate	2.5 g
Agar	15.0 g

pH 7.4 ± 0.2

Additional Ingredients per Liter:

PB54DP BHIA with 10% Sheep Blood & CC

Defibrinated Sheep Blood	100.0 mL
Chloramphenicol	50 mg
Cycloheximide	500 mg

PB55 & TB51 BHIA with 10% Sheep Blood

Defibrinated Sheep Blood	100.0 mL

PB56 & TB52 BHIA with 5% Sheep Blood

Defibrinated Sheep Blood	50.0 mL

PB58DP BHIA with 5% Sheep Blood & CC

Defibrinated Sheep Blood	50.0 mL
Chloramphenicol	50 mg
Cycloheximide	500 mg

Recommended Procedure
(Please refer to appropriate literature for a more detailed procedure)

1. Allow medium to adjust to room temperature prior to inoculation.
2. Inoculate by performing a four-quadrant streak on the plated media to obtain well-isolated colonies. For tubed media, streak the surface of the medium in a fishtail motion from the bottom up.
3. If inoculating a selective medium, it is recommended that a non-selective medium be concurrently inoculated to isolate sensitive strains. All plates should be done in duplicate so that one set can be incubated at room temperature and the other set at 35°C.
4. Incubate aerobically at 35°C. For culturing fungi; it is recommended that duplicates be made with one incubated at room temperature and the other at 35°C.
5. Examine plates and tubes after 18 to 24 hours and at 48 hours. Some fungi may require prolonged incubation for growth therefore keep all plates for 7 days prior to discarding.

Interpretation of Results

After the incubation period examine plates for organisms of interest. When examining primary plates a hand lens or stereoscopic microscope should be available for examining very small colonies. The different types of colonial morphology appearing on the agar plate should be noted as well as the number of each morphotype present. Hemolysis is a useful differential characteristic that is best viewed when a bright light is transmitted from behind the plate.

Additional results such as pigment production and odor should also be recorded. Additional tests should be performed on isolated colonies from pure culture in order to complete identification.

- *Certain pathogenic fungi may be inhibited on selective formulations of BHI therefore inoculation onto a non-selective medium is prescribed*
- *Since nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium*

Quality Control

After checking for correct pH, color, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
BHI Agar	
Streptococcus pneumoniae ATCC 6305	Growth
Pseudomonas aeruginosa ATCC 27853	Growth
BHIA w/ Sheep Blood	
Streptococcus pyogenes ATCC 19615	Growth, β-hemolysis
Streptococcus pneumoniae ATCC 6305	Growth, α-hemolysis
Staphylococcus aureus ATCC 25923	Growth
Escherichia coli ATCC 25922	Growth
Aspergillus niger ATCC 9642	Growth
Candida albicans ATCC 10231	Growth
BHIA w SB, Chloramphenicol & Cycloheximide	
Aspergillus niger ATCC 9642	Growth
Escherichia coli ATCC 25922	Inhibition

Storage and Shelf Life

Our various BHI Agar formulations should be stored away from direct light at 4°C to 8°C. For plated media, the medium side should be uppermost to prevent excessive accumulation of

moisture on the agar surface. Under these conditions the plated mediums have a 12 week shelf life. Our tubed media have the following shelf lives:

TB50-05 – BHIA Slant – 16 weeks
TB50-18 – BHIA Pour Plate – 16 weeks
TB51 – BHIA w 10% Shp Blood Slant – 12 weeks
TB52 – BHIA w 5% Shp Blood Slant – 12 weeks

References

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