

# HHD Broth



Medium used for the differentiation of heterofermentative and homofermentative lactic acid bacteria.

## • CONTENTS (Liter)

Tryptone	10.0 g
Yeast Extract	1.0 g
Casamino Acids	3.0 g
Soy Peptone	1.5 g
Fructose	2.5 g
Sucrose	0.2 g
Potassium Dihydrogen Phosphate	2.5 g
Bromocresol Green	0.066 g
Tween 80	1.0 g
Final pH = 7.0 ± 0.2 at 25°C.	

## • PROCEDURE

Suspend 21.8 G of powder in 1 L of distilled or deionized water. Heat to boiling until completely dissolved. Sterilize by autoclave at 121°C for 15 minutes. Cool to 45-50°C in water bath. Mix well. Dispense in tubes. If necessary, dispense the medium before autoclave.

## • INTERPRETATION

HHD Broth is a medium used for the detection and the differentiation of heterofermentative and homofermentative lactic acid bacteria. The heterofermentative lactic acid bacteria produce CO<sub>2</sub>, lactic acid, acetic acid, ethanol and mannitol from hexoses; the homofermentative lactic acid bacteria produce mainly lactic acid from hexoses. Bromocresol green is the pH indicator that will differentiate the degree of acidification induced by both the groups of bacteria. The Homofermentative bacteria produce a color change of bromocresol green from blue to green, with a blue-green cellular sediment on the bottom of the tube. The heterofermentative bacteria produce a slighter acidification of the substrate without a significant change of the medium color which remains blue with a white cellular sediment. Tryptone, soy peptone, yeast extract and casamino acids provide nitrogen, carbon, vitamins and minerals for growth of microorganisms. Fructose and sucrose are the carbohydrates. Potassium dihydrogen phosphate is the buffering agent. Tween 80 is a surface active agent.

## • TECHNIC

Inoculate the specimen with stab using a sterile needle to the medium. Shake gently for spreading microorganism. Incubate at 30°C for 3 days. Refer appropriate references for recommended test procedure.

## • QUALITY CONTROL FOR USE

### Dehydrated medium

Appearance: free-flowing, homogeneous.

Color: beige.

### Prepared medium

Appearance: clear.

Color: blue.

Incubation conditions: 30°C / 3 days

Microorganism	ATCC	Inoculum CFU	Growth	Characteristics
<i>Lactobacillus fermentum</i>	9338	50-100	good	blue
<i>Lactobacillus delbrueckii</i>	11842	50-100	good	green
<i>Lactobacillus plantarum</i>	8014	50-100	good	green
<i>Escherichia coli</i>	25922	50-100	good	green
<i>Proteus mirabilis</i>	25933	50-100	good	blue

## • STORE

The powder is very hygroscopic. Store the powder at room temperature, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store prepared medium at 2-8°C.

## • REFERENCES

1. Mc Donald, L. C. , Mc Feeters. R. F. Daechel, M. A. Fleming, H.P. (1987). App. Environ. Microbiol. 53, 1382-1384.

## • PACKAGE

Cat. No : MB-H1064 HHD Broth	500 G
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