

**Dihydrolase HiVeg™ Broth Base****MV915**

Dihydrolase HiVeg Broth Base is used for studying dihydrolase reaction of *Vibrio parahaemolyticus*.

**Composition \*\* :**

Ingredients	Grams/Litre
HiVeg peptone	5.0
Yeast extract	6.0
Dextrose	2.0
Sodium chloride	30.0
Bromo cresol purple	0.032

Final pH (at 25°C) 6.8 ± 0.2

\*\* Formula adjusted, standardized to suit performance parameters.

**Directions :**

Suspend 43 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Divide in 2 parts. Add 0.5% L-Arginine to first portion. Use second portion as control. Dissolve completely and dispense 3.0 ml into 13 mm x 100 mm screw cap tube. Sterilize by autoclaving at 10 lbs pressure (115°C) for 15 minutes.

**Principle and Interpretation :**

This medium is prepared by using HiVeg peptone in place of Peptic digest of animal tissue used in the conventional medium. Dihydrolase HiVeg Broth Base is modification of Dihydrolase Broth Base which is formulated as per APHA (1) and is used for studying dihydrolase reaction of *Vibrio parahaemolyticus* from other *Vibrio* species. The medium is supplemented with L-Arginine as a substrate for dihydrolase reaction (2, 3).

L-Arginine is converted to putrescine by the dihydrolase enzyme, however putrescine is also formed from arginine by the decarboxylase system. In the decarboxylase system, L-Arginine undergoes decarboxylation to yield agmatine. Agmatine is then catabolized by the enzyme agmatine dehydrolase to putrescine, CO<sub>2</sub> (Carbon dioxide) and ammonia by way of an intermediate compound monocarbaminyl putrescine (4). It occurs in a two step process. In the first step, hydrolytic removal of NH<sub>2</sub> from arginine takes place by the action of an arginine dihydrolase and arginine desimidase to yield citrulline, ammonia and inorganic phosphate (5). In the second step citrulline undergoes splitting or phosphorelytic cleavage by citrulline ureidase to yield ornithine and carbamylphosphate. Ornithine is then further decarboxylated to putrescine and carbon dioxide. Thus, because of production of amine like putrescine in the medium the pH is elevated (6). Bromo cresol purple is the pH indicator in the medium which turns purple from yellow at alkaline pH. For the confirmation, it is suggested to inoculate into a basal medium tubes which does not contain L-Arginine. Alkalinization of the surface of the medium may be caused by exposure to air, so a dihydrolase negative organism may be misidentified as

**Product Profile :**

Vegetable based (Code MV)©		Animal based (Code M)	
<b>MV915</b>	HiVeg peptone	<b>M915</b>	Peptic digest of animal tissue
<b>Recommended for</b>	:	Studying dihydrolase reaction of <i>Vibrio parahaemolyticus</i> .	
<b>Reconstitution</b>	:	43.0 g/l	
<b>Quantity on preparation (500g)</b>	:	11.62 L	
<b>pH (25°C)</b>	:	6.8 ± 0.2	
<b>Supplement</b>	:	L- Arginine	
<b>Sterilization</b>	:	115°C / 15 minutes.	
<b>Storage</b>	:	Dry Medium - Below 30°C, Prepared Medium 2 - 8°C.	

positive. It is therefore recommended to protect the inoculated tubes from air with a layer of sterile mineral oil. HiVeg peptone and yeast extract provide nitrogenous nutrients to support bacterial growth. Dextrose is the fermentable carbohydrate.

**Quality Control :****Appearance of powder**

Light yellow coloured, may have slightly greenish tinge, homogeneous, free flowing powder.

**Colour and Clarity**

Purple coloured, clear solution without any precipitate.

**Reaction**

Reaction of 4.3% w/v aqueous solution is pH 6.8 ± 0.2 at 25°C.

**Cultural Response**

Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours with added 0.5% L-Arginine.

Organisms (ATCC)	Growth	Arginine dihydrolase
<i>Enterobacter aerogenes</i> (13048)	luxuriant	-
<i>Vibrio cholerae</i> (15748)	luxuriant	-
<i>Vibrio parahaemolyticus</i> (17802)	luxuriant	+

Key : + = purple to yellow to purple  
- = purple to yellow

**References :**

- Speck M.L. (Ed.), 1984, Compendium of Methods for the Microbiological Examination of Foods, 2<sup>nd</sup> ed., APHA, Washington, D.C.
- Moeller V., 1955, Acta Pathol. Microbiol. Scand., 36:158.
- Slade H.D. and Stamp W.C., 1952, J. Bact., 64:455.
- Oginsky E.L. and Gehrig R.F., 1953, J. Biol. Chem., 204:721.
- Sokatch J.R., 1969, Bacterial Physiology and Metabolism, New York : Academic Press, pp. 169.
- MacFaddin J.F., 2000(ed), Biochemical Tests for Identification of Medical Bacteria, 3<sup>rd</sup> edition, Lippincott Williams and Wilkins, New York.