

Reddy's Differential HiVeg™ Agar, Modified (Lactic Streak HiVeg™ Agar) MV926

Reddy's Differential HiVeg Agar Modified is recommended for qualitative and quantitative differentiation of lactic *Streptococci*.

Composition ** :

Ingredients	Grams/Litre
HiVeg peptone	5.0
Papaic digest of soyabean meal	5.0
Yeast extract	5.0
HiVeg extract	5.0
Lactose	1.5
L-Arginine hydrochloride	1.5
Bromo cresol purple	0.002
Sodium carboxymethyl cellulose	10.0
Calcium citrate	10.0
Agar	15.0

Final pH (at 25°C) 6.0 ± 0.2

** Formula adjusted, standardized to suit performance parameters.

Directions :

Suspend 58.0 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (115°C) for 10 minutes.

Principle and Interpretation :

This medium is prepared by using HiVeg peptone and HiVeg extract in place of peptic digest of animal tissue and beef extract respectively that makes the medium free of BSE/TSE risks. This medium is the modification of Reddy's Differential Agar, Modified which was originally described by Reddy et al (1) and further modified by Mullan and Walker (2) and recommended by APHA (3) for the differentiation of lactic *Streptococci*. This medium gives faster results and there is no need of incubation in CO₂ enriched environment. *Lactococcus lactis* and its subspecies *cremoris* and *diacetylactis* are used as starter cultures in dairy products. They are differentiated on the basis of arginine hydrolysis and citrate utilization. Lactose fermenters produce acid and are seen as yellow colonies. Production of acid from lactose causes yellow bacterial colonies of *Lactococcus lactis* subspecies *cremoris*. *Lactococcus lactis* initially produce acid but later on turns to violet-purple colour due to liberated ammonia from arginine. *Lactococcus lactis* subspecies *diacetylactis* produces a more intense purple colour than *Lactococcus lactis*. Citrate utilization is seen as clear zone around the colony.

For quantitative determination, decimal dilution of cultures are prepared and spread on agar plates. After incubation at 36 to 40 hours at 32°C, yellow colonies of subspecies *cremoris* are counted. The plates are further incubated for 4 days and then total count is taken as well as colonies with clear zones of subspecies *diacetylactis* are counted and subtracted from total count to get *Lactococcus lactis* population in the mixture.

Product Profile :

Vegetable based (Code MV) ©	Animal based (Code M)
MV926 HiVeg peptone HiVeg extract	M926 Peptic digest of animal tissue Beef extract

Recommended for : Qualitative and quantitative differentiation of lactic *Streptococci*.

Reconstitution : 58.0 g/l

Quantity on preparation (500g) : 8.62 L

pH (25°C) : 6.0 ± 0.2

Supplement : None

Sterilization : 115°C / 10 minutes.

Storage : Dry Medium - Below 30°C, Prepared Medium 2 - 8°C.

Quality Control :

Appearance of powder

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

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity

Light yellow coloured, opalescent gel forms with greenish tinge in petri plates.

Reaction

Reaction of 5.8% w/v aqueous solution is pH 6.0 ± 0.2 at 25°C

Cultural Response

Cultural characteristics observed after an incubation at 32°C upto 4 days.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Citrate Utilisation	Colour of colony
<i>Lactococcus lactis</i> (8000)	10 ² -10 ³	good-luxuriant	>70%	-	yellow
<i>Lactococcus</i> subsp. <i>cremoris</i> (19527)	10 ² -10 ³	good-luxuriant	>70%	-	purple
<i>Lactococcus</i> subsp. <i>diacetylactis</i>	10 ² -10 ³	good-luxuriant	>70%	+	purple

Key : + = positive, clear zone around the colony

- = negative, no clear zone around the colony.

References :

- Reddy M.S., Vedamuthu E.R., and Reinbold G.W., 1971, Agar medium for differential enumeration of lactic streptococci. Appl. Microbiol., 24 : 947.
- Mullan M.A., and Walker A. L., 1979, Dairy Ind. International, 44:16.
- Downes F.P. and Ito K (Eds.), 2001, Compendium of Methods For The Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.