

**Iron Sulphite HiVeg™ Agar****MV868**

Iron Sulphite HiVeg Agar is recommended for the detection of thermophilic anaerobic organisms causing sulphide spoilage in food.

**Composition \*\* :**

Ingredients	Grams/Litre
HiVeg hydrolysate	10.0
Sodium sulphite	0.5
Iron (III) Citrate	0.5
Agar	15.0

Final pH (at 25°C ) 7.1 ± 0.2

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

**Directions :**

Suspend 26 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Principle and Interpretation :**

This medium is prepared by using Hivveg hydrolysate which is free from BSE/TSE risks associated with animal based peptones. Iron Sulphite HiVeg Agar is the modification of Iron Sulphite Agar which is based on formula of Cameron Sulphite Agar developed by the National Canners Association of America (1). 0.1% sulphite concentration in the original formula was reported by Beerens to be inhibitory to some strains of *Clostridium sporogenes* (2). This observation was later on confirmed by Mossel et al (3), who consequently showed that 0.05% sulphite concentration was not inhibitory to the organisms. For the detection of organisms causing sulphide spoilage either Deep-Shake Culture method or Attenborough and Scarr (4) method may be followed:

In Deep-Shake Culture method, the medium is dispensed in 10 ml amounts in sterile tubes. Sample is inoculated when the medium is at about 50°C and allowed to set. Further incubation is carried out at 55°C for 24-48 hours. Typical thermophilic species of *Desulfotomaculum nigrificans*, produces distinct black spherical colonies in the depth of the medium.

In Attenborough and Scarr (4) method, diluted samples of sugar or any other food to be tested are filtered through membrane filters. These filters are then rolled up and placed in tubes containing just sufficient Iron Sulphite HiVeg Agar (at 50°C) to cover them. The medium is allowed to set and then incubated at 55 - 56°C for 24 - 48 hours. After incubation, the number of black colonies on the membrane filter are counted. This membrane filter technique is quicker, of comparable accuracy and permits the examination of larger samples.

**Note:** The blackening reaction is only presumptive evidence of *Clostridial* growth. Confirmation tests must be carried out to identify the organisms growing in the medium.

**Product Profile :**

Vegetable based (Code MV)☉		Animal based (Code M)
<b>MV868</b>	HiVeg hydrolysate	<b>M868</b> Casein enzymic hydrolysate
<b>Recommended for</b>	: The detection of thermophilic anaerobic organisms causing sulphide spoilage in food.	
<b>Reconstitution</b>	: 26.0 g/l	
<b>Quantity on preparation (500g):</b>	: 19.23 L	
<b>(100g):</b>	: 3.84 L	
<b>pH (25°C)</b>	: 7.1 ± 0.2	
<b>Supplement</b>	: None	
<b>Sterilization</b>	: 121°C / 15 minutes.	
<b>Storage :</b>	: 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**

Yellow coloured, slightly opalescent gel forms in petri plates.

**Reaction**

Reaction of 2.6% w/v aqueous solution is pH 7.1 ± 0.2 at 25°C.

**Cultural Response**

Cultural characteristics observed after an incubation at 55 - 56°C for 24 - 48 hours under anaerobic conditions.

Organisms (ATCC)	Growth	Colour of colony
<i>Clostridium botulinum</i> (25763)	luxuriant	black
<i>Clostridium sporogenes</i> (19404)	luxuriant	black
<i>Desulfotomaculum nigrificans</i> (19858)	luxuriant	black
<i>Escherichia coli</i> (25922)	good	no blackening

**References :**

1. Tanner F.W., 1944, "The Microbiology of Foods", 2<sup>nd</sup> ed., Garrard Press, Illinois, P. 1127.
2. Beerens H., 1958, DSIR, Proc. 2<sup>nd</sup> Internat. Sym. Food Microbiol., 1957, HMSO, London, P. 235.
3. Mossel D.A.A., Golstein Brouwers G.W.M.V. and de Bruin A.S., 1959, J. Path. Bact., 78:290.
4. Attenborough J. and Scarr M., 1957, J. Appl. Bact., 20:460.