



Spirit Blue HiVeg™ Agar

MV445

Spirit Blue HiVeg Agar is used for detection and enumeration of lipolytic microorganisms.

Composition**

Ingredients	Gms / Litre
HiVeg hydrolysate	10.000
Yeast extract	5.000
Spirit blue	0.150
Agar	17.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.15 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. Cool to 50°C and add 30ml lipase substrate slowly while agitating to obtain an even distribution.

Note : For proper lipase activity, it is recommended to use glass plates instead of disposable plastic plates.

Principle And Interpretation

Spirit Blue HiVeg Agar is prepared by completely replacing animal based peptones with vegetable peptones that makes the medium free of BSE/TSE risks. It can be used for the same purpose of Spirit Blue Agar which is prepared according to the formulation of Starr (1) and is recommended by APHA (2) for the detection, enumeration and study of lipolytic microorganisms. Lipids, including fats and oils, are highly reduced. When a lipid is catabolized, it has the potential to yield more pairs of electrons per gram, and thus more energy, than either carbohydrates or proteins (3). This process is brought about by the enzyme lipase, and the organisms possessing this enzyme are called lipolytic organisms. Growth of lipase producing microorganisms can contribute to flavour defects in milk and high fat dairy products. Some of the free fatty acids released by the action of lipolytic enzymes have a low flavour threshold and can impart a rancid flavour at low concentrations. It is a basal medium to which lipoidal substrate is added for the detection, enumeration and study of lipolytic microorganisms. Formulations in practice before Starr which included dyes as indicators of lipolysis were sometimes inhibitory to the microorganisms. Starr showed spirit blue to be inert and an ideal indicator of lipolysis, visualized as clear halos around colonies. HiVeg Hydrolysate and yeast extract are the sources of carbon, nitrogen, vitamins and minerals. Spirit blue is the indicator of lipolysis. The lipase reagents recommended as the lipid source are cotton seed meal, cream, olive oil etc. A satisfactory emulsion can be prepared by dissolving 10 gram acacia or 1 ml polysorbate 80 in 400 ml warm distilled water, adding 100 ml cotton seed or olive oil and agitating vigorously to emulsify. Prepare 1:10 or other suitable dilution of the product to be tested. Spread 0.1 ml of the desired dilutions over the surface of the medium. Incubate at 35-37°C for 24-48 hours. Colonies of lipolytic organisms develop a clear zone and /or a deep blue colour around and under each colony (2).

Quality Control

Appearance

Yellow to greyish yellow Homogeneous Free flowing powder

Gelling

Firm, comparable with 1.7% agar gel.

Colour and Clarity of prepared medium

Basal:Blue After addition of lipase substrate : Lavender Basal : Clear to slightly opalescent After Addition: opaque

Reaction

Reaction of 3.22% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed with added Lipase substrate after an incubation at 35 - 37°C for 48 - 72 hours.

Cultural Response

Organism	Growth	Lipase activity
Cultural Response <i>Proteus mirabilis</i> ATCC 25933	luxuriant	Negative, absence of zone around colony
<i>Staphylococcus aureus</i> ATCC 25923	luxuriant	Positive, clear zone around colony
<i>Staphylococcus epidermidis</i> ATCC 12228	luxuriant	Positive, clear zone around colony

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

- 1.Starr. 1941. Science, 93.
- 2.Downes, F.P. and Ito, K. 2001. Methods For The Microbiological Examination of Foods. APHA, Food 4 ed. Washington, D.C.
- 3.Norton, C. F. 1986. Microbiology. 2 ed.: Addison-Wesley Publishing Company.

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