Technical Information

Spirit Blue MiVeg Agar

Product Code : VM1445

Application:- Spirit Blue MiVeg Agar is used for detection and enumeration of lipolytic microorganisms.

Composition**

Ingredients	Gms / Litre
MiVeg 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.

Principle & Interpretation

Spirit Blue MiVeg Agar is prepared by using MiVeg hydrolysate in place of Casein enzymic hydrolysate thereby making the the medium BSE/TSE risks free. This medium is the modification of Spirit Blue Agar which is developed as per formula of Starr (1) and is also recommended by APHA (2) for the detection, enumeration and study of lipolytic microorganisms. Lipids, including fats and oils, are highly reduced. Lipid catabolism yields more pairs of electrons per gram, and thus more energy, than either carbohydrates or proteins (3). This entire process is brought about by the enzyme lipase, and the organisms possessing this enzyme are called lipolytic organisms. Growth of lipase producing microorganisms imparts 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 contribute a rancid flavour at low concentrations. Formulations in practice before Starr that included dyes as indicators of lipolysis were sometimes inhibitory to the microorganisms. Starr introduced spirit blue, an inert and an ideal indicator of lipolysis, visualized as clear halos around colonies.

MiVeg Hydrolysate and yeast extract are the sources of carbon, nitrogen, vitamins and minerals. Spirit blue act as a lipolysis indicator. 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 with vigorous agitation to emulsify. Prepare 1:10 or other suitable dilution of the product under examination. 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 as a clear zone and /or a deep blue colour around and under each colony (2).

Methodology

Suspend 32.15 grams of powder media in 1000 ml distilled water. Mix thoroughly and 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 : To study proper lipase activity, it is recommended to use glass plates instead of disposable plastic plates.

Quality Control

Physical 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 is pH 6.8±0.2 at 25°C.

pH Range

6.6-7.0

Cultural Response/Characteristics

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

Organisms (ATCC)	Growth	Lipase activity
Proteus mirabilis ATCC 25933	luxuriant	Negative, absence of zone around colony
Staphylococcus aureus ATCC 25923	luxuriant	Positive, clear zone around colony
Staphylococcus epidermidis ATCC 12228	luxuriant	Positive, clear zone around colony

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 day.

Further Reading

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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