TM 1409 - SPIRIT BLUE AGAR

INTENDED USE

For detection and enumeration of lipolytic microorganisms.

PRODUCT SUMMARY AND EXPLANATION

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. This process is brought about by the enzyme lipase, and the organisms possessing the enzyme lipase 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.
Spirit Blue Agar is prepared according to the formulation of Starr is recommended by APHA for detection and enumeration of lipolytic microorganisms. 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.

COMPOSITION

Ingredients	Gms / Ltr
Casein enzymic hydrolysate	10.000
Yeast extract	5.000
Spirit blue	0.150
Agar	17.000

PRINCIPLE

Casein enzymic hydrolysate and yeast extract in the medium are sources of carbon, nitrogen, vitamins and minerals. Spirit blue is a dye which acts as an 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 gm 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.

INSTRUCTION FOR USE

- Dissolve 32.15 grams in 1000 ml distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 50°C and add 30 ml 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.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to greenish yellow homogeneous free flowing powder.
Appearance of prepared medium : Basal medium yields blue coloured, clear to slightly opalescent gel. With addition of lipase substrate, lavender coloured slightly opalescent gel forms in Petri plates.
pH (at 25°C) : 6.8±0.2

INTERPRETATION

Cultural characteristics observed after an incubation with added Lipase substrate.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Lipase activity	Recovery	Incubation Temperature	Incubation Period
Proteus mirabilis	25933	50-100	Luxuriant	Negative, absence of zone around colony	>=70%	35-37°C	48-72 Hours
Staphylococcus aureus	25923	50-100	Luxuriant	Positive reaction, clear zone around colony	>=70%	35-37°C	48-72 Hours
Staphylococcus epidermidis	12228	50-100	Luxuriant	Positive reaction, clear zone around colony	>=70%	35-37°C	48-72 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

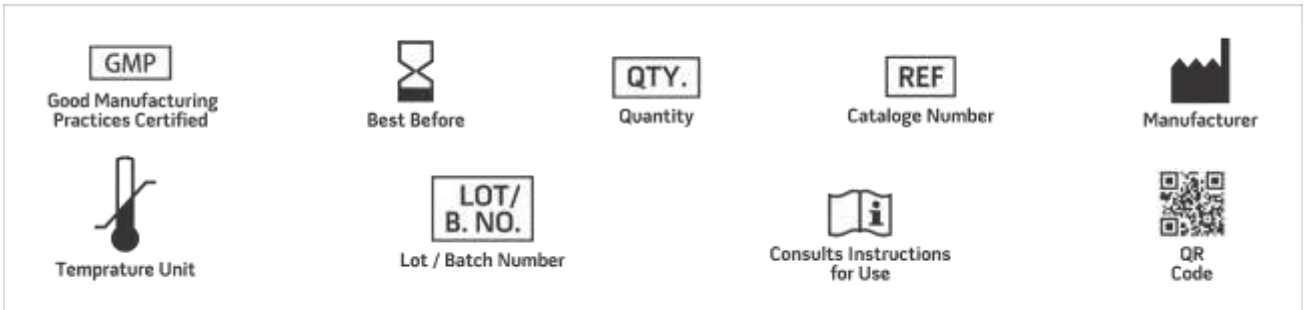
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. Nortan C. F., 1986, Microbiology, 2nd Ed., Addison-Wesley Publishing Company.
2. Starr, 1941, Science, 93:333.3. Marshall R. T., (Ed.) 1993, Standard Methods for the Examination of Dairy Products, 16th Ed, APHA, Washington, D.C.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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