

Spirit Blue Agar Lipase Reagent

Intended Use

Spirit Blue Agar is for use with Lipase Reagent or other lipid source for detecting and enumerating lipolytic microorganisms.

Summary and Explanation

In 1941, Starr¹ described a lipid emulsion medium for detecting lipolytic (lipase-producing) microorganisms to which he added the dye, spirit blue. Other dyes used as indicators of lipolysis were toxic to many microorganisms. Spirit blue did not have toxic effects. When testing samples of dairy products, air and sewage on Spirit Blue Agar, Starr obtained accurate counts of lipolytic microorganisms and total microbial counts on the same medium.

Lipolytic microorganisms, such as psychrotrophic bacteria, molds or yeasts, can adversely affect the flavor of milk and high fat dairy products. Spirit Blue Agar is a recommended medium

for testing milk and dairy products.²

Lipase Reagent, a mixture of tributyrin and polysorbate 80, is recommended as the lipid source. Other lipoidal emulsions may be prepared from cottonseed meal, cream, vegetable oil and olive oil. A satisfactory emulsion can be prepared by dissolving 10 g gum acacia or 1 mL polysorbate 80 in 400 mL warm purified water, adding 100 mL cottonseed or olive oil and agitating vigorously to emulsify.

Principles of the Procedure

Spirit Blue Agar contains peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Spirit blue is the indicator of lipolysis. Agar is the solidifying agent.

User Quality Control

Identity Specifications

Difco™ Spirit Blue Agar

Dehydrated Appearance: Grayish-beige, free-flowing, homogeneous.

Solution: 3.5% solution, soluble in purified water upon boiling. Solution is royal blue, slightly opalescent.

Prepared Appearance: Plain – Royal blue, opalescent.
With 3% Lipase Reagent – Pale blue, opalescent.

Reaction of 3.5%
Solution at 25°C: pH 6.8 ± 0.2

Difco™ Lipase Reagent

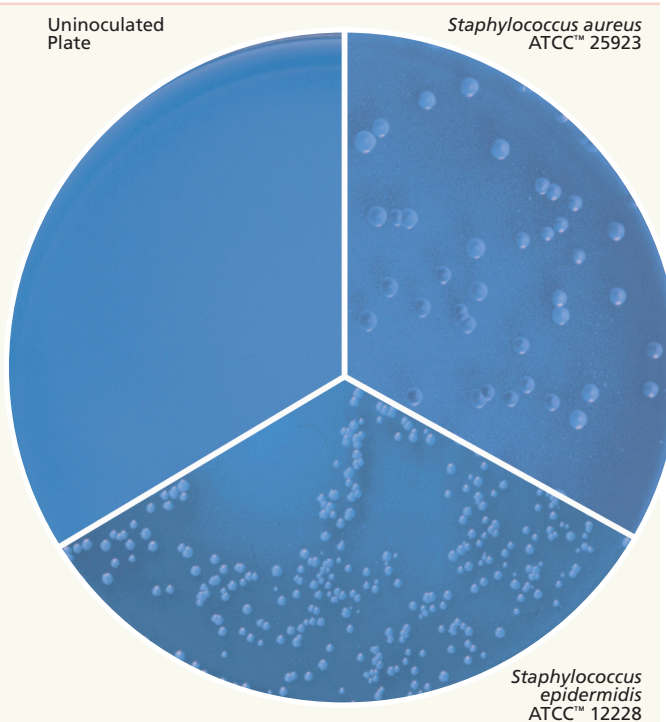
Appearance: White, opaque emulsion.

Cultural Response

Difco™ Spirit Blue Agar and Lipase Reagent

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for up to 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HALO/ LIPOLYSIS
<i>Proteus mirabilis</i>	25933	10 ² -10 ³	Good	–
<i>Staphylococcus aureus</i>	25923	10 ² -10 ³	Good	+
<i>Staphylococcus aureus</i>	6538	10 ² -10 ³	Good	+
<i>Staphylococcus epidermidis</i>	12228	10 ² -10 ³	Good	+



Lipase Reagent contains tributyrin, a true fat and the simplest triglyceride occurring in natural fats and oils. It is a good substrate when testing for lipolytic microorganisms because some microorganisms that hydrolyze tributyrin will not hydrolyze other triglycerides or fats containing longer chain fatty acids.²

Formulae

Difco™ Spirit Blue Agar

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	10.0 g
Yeast Extract	5.0 g
Agar	20.0 g
Spirit Blue	0.15 g

Difco™ Lipase Reagent

A ready-to-use lipid suspension, containing a mixture of tributyrin and polysorbate 80.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 35 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 50-55°C.
4. Aseptically add 30 mL Lipase Reagent or other lipid source and mix thoroughly.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the organism onto the medium. Incubate plates at $35 \pm 2^\circ\text{C}$ for up to 72 hours, or at other temperatures and times according to standard methods.²

Expected Results

Lipolytic microorganisms metabolize the lipid in the medium and form colonies with halos indicating lipolysis.

References

1. Starr. 1941. Science 93:333.
2. Frank and Yousef. 2004. In Wehr and Frank (ed.), Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Spirit Blue Agar

SMD

Cat. No. 295010 Dehydrated – 100 g
295020 Dehydrated – 500 g

Difco™ Lipase Reagent

SMD

Cat. No. 243110 Bottle – 6 × 20 mL