

TSN Agar

Intended Use

TSN (Trypticase™ Sulfite Neomycin) Agar is used for the selective isolation of *Clostridium perfringens*.

User Quality Control

Identity Specifications

BBL™ TSN Agar

Dehydrated Appearance: Fine, homogeneous, may contain a large amount of minute to small dark particles.

Solution: 4.0% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to moderately hazy.

Prepared Appearance: Light to medium, yellow to tan, clear to moderately hazy.

Reaction of 4.0%

Solution at 25°C: pH 7.2 ± 0.2

Cultural Response

BBL™ TSN Agar

Prepare the medium per label directions. Inoculate and incubate at 46 ± 1°C anaerobically for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	APPEARANCE
<i>Clostridium bifermentans</i>	17836	Undiluted	Partial to complete inhibition	With or without blackening
<i>Clostridium perfringens</i>	3624	Undiluted	Good	Blackening
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Enteritidis	13076	10 ⁴ -10 ⁵	Partial to complete inhibition	No blackening

Summary and Explanation

TSN Agar was developed by Marshall et al. as a medium that could achieve rapid enumeration of *Clostridium perfringens*.¹ The formulation is a modification of Mossel's medium for the enumeration of sulfite-reducing clostridia in foods.² The 46°C temperature of incubation for TSN Agar permits specific and quantitative results.

Principles of the Procedure

Neomycin and polymyxin are inhibitory for gram-negative enteric bacilli. Neomycin at the concentration employed at least partially inhibits *C. bifermentans*. The relatively high incubation temperature of 46°C renders the medium highly specific for *C. perfringens*. The colonies are black due to the formation of ferric sulfide as a result of the reduction of the sulfite.

Formula

BBL™ TSN Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Sodium Sulfite.....	1.0	g
Neomycin Sulfate	0.05	g
Polymyxin Sulfate	0.02	g
Yeast Extract	10.0	g
Ferric Citrate	0.5	g
Agar	13.5	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 40 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. Dispense and autoclave at 118°C for 12 minutes. Do not overheat.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use on the day of preparation. Inoculate tubes or plates of the medium by stabbing deep tubes or streaking plates with the test specimen. Incubate containers for 18-24 hours at 46 ± 0.1°C in an anaerobic atmosphere (BD GasPak™ EZ anaerobic system or equivalent).

Expected Results

C. perfringens produces black colonies at 46°C. *C. perfringens* and *C. bifermentans* produce black colonies on TSN Agar at 37°C; however, *C. bifermentans* is inhibited at 46°C.¹

References

1. Marshall, Steenbergen and McClung. 1965. Appl. Microbiol. 13:559.
2. Mossel. 1959. J. Sci. Food Agric. 10:662.

Availability

BBL™ TSN Agar

Cat. No. 211690 Dehydrated – 500 g