

Sulfite Polymyxin Sulfadiazine Agar, SPS Agar (i23173)

Medium proposed by ANGELOTTI et al. (1962) for the isolation and enumeration of *Clostridium perfringens* and *Clostridium botulinum* in all types of foodstuffs. Industry: Food

Principles & Uses

Sulfite Polymyxin Sulfadiazine Agar (SPS Agar) -also known as Perfringens Selective Agar acc. to ANGELOTTI- serves as a moderately selective medium for the isolation of *Clostridium perfringens* from various food sources. Developed with a base similar to the Wilson and Blair Medium, this modified SPS Agar includes sulfadiazine and polymyxin B sulfate, effectively inhibiting a wide range of grampositive and gram-negative bacteria. The blackening of colonies on the medium is a result of sulfite reduction by most *clostridia*, reacting with ferric citrate.

The composition includes pancreatic digest of casein and yeast extract, supplying essential nutrients for *C. perfringens* growth. Ferric citrate and sodium sulfite act as indicators, forming a black precipitate in the presence of sulfide produced by *C. perfringens*. Polymyxin B sulfate and sulfadiazine prevent the growth of organisms other than *Clostridium* spp. Agar solidifies the medium.

To recover *C. perfringens*, serial dilutions of samples are inoculated onto SPS Agar using the pour plate technique. The black colonies, indicative of sulfite reduction, are enumerated, and presumptive *C. perfringens* colonies are confirmed through standard biochemical tests. Despite some growth by organisms other than *C. perfringens*, the distinctive characteristics aid in accurate identification.

Composition (gr/L)

Pancreatic digest of Casein 15 g, Yeast Extract 10 g, Ferric Citrate 0.5 g, Sodium Sulfite 0.5 g, Sulfadiazine 0.12 g, Polymyxin Sulfate 0.01 g, Agar 13.9 g. Final pH at 25° C 7.0 ± 0.2

Preparation from dehydrated Powder

Suspend 40 g in 1 L of distilled water. Autoclave for 15 min at 121°C.

Quality Control

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Prepared Appearance: Light to medium amber, slightly opalescent.

Reaction of 4.0% Solution at 25°C: pH 7.0 ± 0.2

Cultural Response

Inoculate using the pour plate technique and incubate anaerobically at $35 \pm 2^{\circ}C$ for 24 - 48 hours.

Organism (ATCC*)	Recovery	Colony color
Clostridium perfringens (12919)	Good	Black
Clostridium sporogenes (11437)	None to fair	Black
Escherichia coli (25922)	Marked to complete inhibition	-
Salmonella enterica (14028)	Marked to complete inhibition	-
Staphylococcus aureus (25923)	Fair to good	White

*ATCC is a registered trade mark of the American Type Culture Collection.



Clostridium perfringens (12919)

Storage

Keep the container at 15-30 °C. Store prepared medium at 2-8 °C.