

SPS AGAR (SULFITE POLYMYXIN SULFADIAZINE)

CAT Nº: 1082

For the isolation and enumeration of *Clostridium perfringens* from food and all types of foodstuffs

FORMULA IN g/l

Caseine Peptone	15.50	Sulfadiazine	0.12
Yeast Extract	10.00	Polymyxin B Sulfate	0.01
Ferric Citrate	0.50	Bacteriological Agar	13.00
Sodium Sulfite	0.50		

Final pH 7.0 ± 0.2 at 25°C

PREPARATION

Suspend 39.7 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45-50°C, mix well and dispense into plates. The prepared medium should be stored at 8-15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

SPS AGAR (Sulfite Polymyxin Sulfadiazine Agar) is a moderately selective medium to recover *Clostridium perfringens* from fresh or preserved foods and food ingredients.

The medium was modified by Angelotti, incorporating Sulfadiazine and Polymyxin B sulphate to the more recent Mossel formula for the recovery of *Clostridium perfringens*.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Ferric citrate and Sodium sulfite are H₂S indicators. *C. perfringens* reduces the sulfite to sulfide which in turn reacts with the iron and forms a black iron sulfide precipitate, seen as black colonies.

Material samples are prepared in a homogenizer, or other equipment, and serial dilutions are plated in SPS Agar, previously cooled to 45-50°C. Incubate anaerobically. (The authors used a mixture of 90% nitrogen and 10% CO₂). Serial dilutions in tubed media can also be made and incubated aerobically at 35 ± 2°C for 24-48 hours.

A few microorganisms other than *C. perfringens* also grow on SPS Agar so it is best to perform a Gram stain and look for spores. Many common microorganisms are totally or partially inhibited, but if they develop, they generally do not form black colonies nor spores, nor do they reduce nitrate and are non-motile Gram-positive vegetative bacilli.

The lack of motility and the capacity to reduce nitrate can be determined on Indole Nitrate Medium (Cat. 1504) with 2 g/l of added agar.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 24-48 hours.

Microorganisms	Growth	Colony Color
<i>Clostridium perfringens</i> ATCC 13124	Good	Black
<i>Clostridium sporogenes</i> ATCC 11437	Moderate	Black
<i>Escherichia coli</i> ATCC 25922	Inhibited	
<i>Staphylococcus aureus</i> ATCC 6538	Moderate inhibited	White

BIBLIOGRAPHY

Angelotti, Nall, Foter y Lewis. Applied Microbiol. 10: 193. 1962. Mossel. J.SCI. Agr. 10: 662. 1959. Mossel de Bruin Van Diepen, Vendrig y Zoutwelle J. Applied Bact, 19: 142. 1956.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

