

17231 SPS Agar, modified (Sulfite Polymyxin Sulfadiazine Agar, modified) NutriSelect® Plus

For the selective isolation and enumeration of *Clostridium perfringens* from foods.

Composition:

Ingredients	Grams/Litre
Casein enzymic hydrolysate	15.0
Yeast extract	10.0
Ferric citrate	0.5
Sodium thioglycollate	0.1
Sodium sulfite	0.5
Polysorbate 80	0.05
Sulfadiazine	0.12
Polymyxin B sulfate	0.01
Agar	15.0

Final pH 7.0 +/- 0.2 at 25°C

Store dehydrated powder between 2-8°C in a tightly closed container. Use before expiry date on the label.

Appearance(color): Faint Yellow, faint beige to faint brown, Free flowing powder

Gelling: Firm, comparable with 1.5% Agar gel.

Color and Clarity: Medium amber coloured slightly opalescent gel forms in Petri plates.

Directions:

Suspend 41.28 g in 1 litre distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour in sterile petri plates containing inoculum. Allow to solidify and if desired, pour the cover layer using about 5 ml sterile medium. Incubate anaerobically.

Principle and Interpretation:

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

It was developed by Angelotti et al (1) based on the Wilson and Blair Medium and the medium described by Mossel et al (2, 3) for selective isolation and enumeration of *C. perfringens* from foods. The medium of Mossel et al included the use of Miller-Prickett tubes. The modified SPS Agar however obviates the inclusion of Miller-Prickett tubes.

Casein enzymic hydrolysate and yeast extract supply nitrogenous compounds, vitamin B complex and other essential growth nutrients to the growing *C. perfringens*. Ferric citrate and sodium sulfite are H₂S indicators. *C. perfringens* reduces the sulfite to sulfide which in turn reacts with the iron of ferric citrate to form a black iron sulfide precipitate, seen as black colonies (4). Polymyxin B and sulphadiazine inhibit a wide variety of gram-positive and gram-negative bacteria (5). Few organisms found in food other than *C. perfringens* also form black colonies on this medium.

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.



Cultural characteristics observed after an incubation of 18- 48 hours at 35°C under anaerobic conditions

Organisms (ATCC/WDCM)	Inoculum (CFU)	Growth	Recovery	Color of colony
<i>Clostridium perfringens</i> (12924/-)	50-100	+++	≥50%	Black
<i>Clostridium sporogenes</i> (19404/00008)	50-100	++	30-40%	Black
<i>Escherichia coli</i> (25922/00013)	>=10 ³	-	0%	-
<i>Staphylococcus aureus</i> (6538/00032)	50-100	+/-	≤10%	White

References:

1. Angelotti R., Han H. E., Foter M. J. and Lewis K. H., 1962, *Appl. Microbiol.*, 10:193.
2. Mossel D. A. A., De Bruit A. S., Van Dipen H. M. J., Vendring C. M. A. and Zoutewelle G., 1956, *J. Appl. Microbiol.*, 19:142.
3. Mossel R. S., 1959, *J. Sci. Food Agric.*, 19:662.
4. Downes F. P. and Ito K., (Eds.), 2001, *Compendium of Methods for the Microbiological Examination of Foods*, 4th Ed., APHA, Washington, D.C
5. MacFaddin J. F., 1985, *Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria*, Vol. 1, Williams and Wilkins, Baltimore

Precautions and Disclaimer

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