

## INTENDED USE

PRO-LAB *Haemophilus* Selective Supplement is an antibiotic and nutrient medium supplement used to enhance the isolation of *Haemophilus sp.*

## SUMMARY AND EXPLANATION

Haemophili are non-motile, Gram-negative organisms which are strictly parasitic and, when being cultured in vitro, require growth factors present in blood, especially X (Hemin) and/or V(NAD) factor. In culturing *Haemophilus sp.*, difficulties are often encountered due to the overgrowth by more strongly growing commensal organisms.

Two groups Crawford, Barden and Kirkman<sup>1</sup> and Klein and Blazevic<sup>2</sup> reported the development of a medium for the selective isolation of *Haemophilus sp.* using bacitracin as a selective agent. It supported the growth of *Haemophilus sp.* while suppressing growth of all Gram-positive bacteria and *Neisseria sp.* More recent work by Tempro and Slots<sup>3</sup>, confirmed the addition of bacitracin (75 mg/l) to the culture medium inhibits the growth of most commensal organisms resulting in a high recovery rate of *Haemophilus sp.* The addition of hemin, NAD and menadione to the culture medium enhances the growth of *Haemophilus sp.*

## DESCRIPTION

Accurate quantities of Bacitracin, NAD, Hemin and Menadione are lyophilized and provided in individually labelled vials, each vial being sufficient to supplement 1000 mls of prepared medium.

## FORMULA

Each vial contains:

Bacitracin	75 mg
NAD	12.5 mg
Hemin	1.2 mg
Menadione	2.4 mg

## PROCEDURE

To reconstitute each vial of PRO-LAB *Haemophilus* Selective Supplement, add, aseptically, 10 ml of sterile distilled water. After reclosing the vial, gently agitate to assist reconstitution.

Prepare Blood Agar according to the manufacturer's instructions, autoclave and cool to 55°C. Add 5-7% sterile, defibrinated horse blood, and mix thoroughly. Heat to 80°C, mixing occasionally, until the medium becomes a chocolate brown colour. Cool medium to 50°C. Add the reconstituted contents of one vial of PRO-LAB *Haemophilus* Selective Supplement PL.430S to a final 1000 ml prepared medium. Mix gently and pour into sterile petri dishes. Overnight storage at 4°C is recommended to allow suitable equilibration. For extended storage at 4°C, eg. up to 7 days, plates should be contained in sealed plastic sleeves or similar packaging.

## IN USE

1. Before using selective medium ensure that plates are dry.
2. Inoculate test material onto surface of agar using sterile inoculating loop (available from PRO-LAB) or a sterile swab in such a manner as to encourage the growth of isolated colonies.
3. Incubate plates at 37°C in an atmosphere containing 10% CO<sub>2</sub>.
4. Examine after incubation (18-24 hours).
5. Suspect colonies of *Haemophilus sp.* should be further tested for X and V factor requirement.

## SAFETY PRECAUTIONS

1. PRO-LAB *Haemophilus* Selective Supplement PL.430S is offered only as an in vitro material and is in no way intended for a curative or prophylactic purpose.
2. During and after use, handle all materials in a manner conforming to Good Laboratory Practices and consider at all times that material under test should be regarded as a potential biohazard if mishandled.

## PRESENTATION

PRO-LAB *Haemophilus* Selective Supplement PL.430S is supplied 10 vials per box (lyophilized).









## STORAGE

PRO-LAB *Haemophilus* Selective Supplement PL.430S must be stored at 2°C to 8°C. Kept under these conditions it may be stored up to date of expiry shown on product label.

## REFERENCES

1. Crawford, J.J., Barden, L., and Kirkman, J.B. 1969. Appl. Microbiol. 18: 646-649.
2. Klein, M., and Blazevic, D.J. 1970. Am. J. Med. Tech. 36: 97-106.
3. Tempro, P.J., and Slots, J. 1986. J. Clin. Microbiol. 23: 777-782.

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	= Use by
	= Lot number
	= Catalogue number
	= Manufacturer
	= Authorized Representative in the European Community.
	= in vitro diagnostic medical device.
	= Temperature limitation
	= Consult instructions for use.