



PORPHYRIN (ALA SUBSTRATE)

-For in vitro use only-

Catalogue No. TP83

Our Porphyrin (ALA Substrate) tubes are used to differentiate *Haemophilus* species based on their ability to synthesize heme and its precursors. Porphyrin tubes can replace conventional satellite X-factor tests with the added benefit of increased accuracy and rapidity of results.

Our Porphyrin tubes contain δ -aminolevulinic acid (ALA) which is the precursor for porphobilinogen, porphyrins, and heme. Most *Haemophilus* species require exogenous X factor (hemin) for growth, but certain strains of *Haemophilus* which possess the enzyme porphobilinogen synthase are hemin-independent and can synthesize their own heme. *Haemophilus* species with the enzyme will excrete different by-products including porphobilinogen and porphyrins. Detection of these by-products is possible since many porphyrins (i.e. uroporphyrin, copro-porphyrin, protoporphyrin) emit a strong red fluorescence when illuminated by ultraviolet light.

An alternative method for detection of porphyrins is the addition of Kovac's Reagent, which produces a pink end-product if porphyrins are present.

Formula per Litre of Medium

Disodium phosphate7.9 g
Monopotassium Phosphate.....6.1 g
 δ -Aminolevulinic acid0.34 g
Magnesium sulfate.....0.096 g

pH 6.9 \pm 0.2

Recommended Procedure

1. Allow the medium to adjust to room temperature.

- Using a very heavy inoculum taken from an 18-24 hour pure culture of suspected *Haemophilus* species inoculate the Porphyrin tube.
- Incubate tube aerobically at 35°C for 4-6 hours.
- After incubation, expose the inoculated substrate tube to an UV (360nm) source in a darkened room or black box and observe for fluorescence.
- If an UV source is not available, add 0.5 mL of Kovac's Reagent (Dalynn RK75) to the tube and mix vigorously. Wait 5 minutes to allow phases to separate and interpret.

Interpretation of Results

Positive: Red fluorescence under UV light or the development of a pink color after the addition of Kovac's Reagent indicates that porphyrins are present and that the *Haemophilus* strain **does not** require exogenous X factor

Negative: No fluorescence under UV light or no color change (yellow) after the addition of Kovac's Reagent indicates that porphyrins are not present and that the *Haemophilus* strain **does** require exogenous X factor

- Cultures being tested must not be older than 24 hours*
- The inoculum must be heavy otherwise the 4-6 hour incubation period may be inadequate and result in false negatives*

- *Test only Haemophilus species since other bacteria commonly found in the oropharynx can make heme and yield false-positive results*
- *The fluorescence test has been shown to be superior to the use of Kovac's reagent due to its increased sensitivity*

4. Gadbury JL, Amos MA. Comparison of a new commercially prepared porphyrin test and the conventional satellite test for the identification of *Haemophilus* species that require the X factor. *J Clin Microbiol* 1986; 23(3):637-9.
5. McFaddin JF, MacFaddin, JF. *Biochemical Tests for the Identification of Medical Bacteria*, 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

Quality Control

After checking the medium for correct pH, colour, depth, slant and sterility, the following organisms are used to determine the performance of the completed medium.

<u>Organism</u>	<u>Expected results</u>
<i>Haemophilus parainfluenzae</i> ATCC 7901	+ Red fluorescence
<i>Haemophilus influenzae</i> ATCC 10211	- No fluorescence

Original: January 2005
Revised / Reviewed: October 2014

Storage and Shelf Life

Our Porphyrin (ALA Substrate) tubes should be stored in an upright position at -20°C and protected from light. Under these conditions the substrate has a shelf life of 26 weeks from the date of manufacture.

References

1. Biberstein EL, Mini PD, Gills MG. Action of *Haemophilus* cultures on δ -aminolevulinic acid. *J Bact* 1963; 86:814-9.
2. Kilian M. A rapid method for the differentiation of *Haemophilus* strains. *Acta Pathol Microbiol Scand* 1974; 82:835-42.
3. Lund ME, Blazevic DJ. Rapid speciation of *Haemophilus* with the porphyrin production test versus the satellite test for X. *J Clin Micro* 1977; 5:142-4.