

## SWAB-IT PO4 / PROLINE

Some bacteria produce enzymes during the growth process and because the presence of the enzymes can be detected rapidly, more substrates can be used in a wide variety of applications which would normally require special growth conditions.

PO4: When bound to nitrophenol, the hydrolysis of the colorless aryl-substituted glycoside releases the nitrophenol base with its yellow color. Enzymatic hydrolysis of the PRO arylamide in the swab releases free  $\beta$ -naphthylamine which is detected and shown by the color change after adding reagent.

### ACTIVE INGREDIENTS:

The swabs contain L-Proline- $\beta$ -naphthylamide and p-Nitrophenol phosphate (PO4). MSDS for the substrates are available at [Keyscientific.com](http://Keyscientific.com).

### MATERIAL REQUIRED:

The test requires fresh 24 hour growth on plated media appropriate for the specimen. Swab-it PO4/PRO is provided in packs of 25 with developer & hydrator included.

### INSTRUCTIONS:

(1) Lightly pick up several isolated colonies with the dry swab, taking care not to pick up agar. Harvest enough colonies to see a paste on the swab.

(2) Drop the swab in a tube containing 3 drops of hydrating fluid. Incubate aerobically for 2 hours at 32-37C.

### INTERPRETATION:

PO4: No change is negative. The appearance of a bright yellow color is a positive test-pale color is negative. After reading the PO4 results add 2 drops of PEP reagent. Incubate 5 minutes for color development. Positive tests will be red while negative tests are yellow to a very light peach color.

**LIMITATIONS:** Indole reactions of organisms grown on any media containing tryptophane may interfere with the PRO reaction. From such media, positive reactions may be red (naphth+/indol-) or very dark blue to purple (naphth+/indole+). Green or turquoise (indole+), and yellow (indol-) are PRO negative. Some organisms also contain enzymes which react with PEP reagent to produce a red color not created by the PRO chemical. A negative control may be done by streaking a piece of filter paper with the organism and adding reagent.

### STORAGE:

Store swabs and developer, tightly sealed, at 2-8C. All items may be used cold.

### QUALITY CONTROL:

Each lot should be tested with known positive and negative organisms. Key suggests *Serratia marcescens* ATCC 8100 Proline and PO4 positive, and *Lactobacillus casei* ATCC 393 negative for both.

### REFERENCES

(1) Methods in Microbiology, 1976. Chapter 1, Substrate Specificities of Aminopeptidases: A specific Method for Microbial Differentiation

(2) Manual of Clinical Microbiology, Fifth Edition, Chapter 29, Streptococcus.



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