

IDENTICULT-PRO (Proline)

CAT. NO. KF4020 - 50/KIT TEST CARDS

PURPOSE: Identicult-PRO is recommended for use in screening suspected *Clostridium difficile* isolates for L-proline aminopeptidase activity and a lack of indole activity.

PRINCIPLE: Enzymatic hydrolysis of L-proline- β -naphthylamide releases free- β naphthylamine which is shown by the color change after adding PEP reagent. This test is especially useful in screening for *Clostridium difficile*. A negative test indicates the organism cannot be *C. difficile*. The test is also of value when used with other tests in the speciation of many anaerobes and other organisms such as enterics. Comprehensive charts for these organisms are available on request.

ACTIVE INGREDIENTS/ MSDS:

The Identicult-PRO contains L-proline β -naphthylamide. Naphthylamides have been identified as possible carcinogens however when used as directed the Identicult present no hazard.

CONTENTS: Reagent #1 Buffer 15 ml, Reagent #2 Color Developer 15 ml, IDENTICULT-PRO CARDS 50 (5 x10).

MATERIALS REQUIRED BUT NOT PROVIDED:

Standard microbiological supplies and equipment such as loops, needles, and wooden sticks are not provided.

PRECAUTIONS: Precautions should be taken against the dangers of microbiological hazards. Do not touch the strips prior to or after the addition of organisms or reagent. Use of this test should be limited to individuals with appropriate bacteriological training. Sterilize prior to disposal.

STORAGE: Upon receipt store IDENTICULT-PRO cards at 2-8°C. Opened bags should be resealed to reduce the possibility of moisture accumulation. Allow refrigerated product to warm to room temperature before use. Do not use this product if the paper disk is not white or if the expiration date has passed. Do not freeze the reagent.

LIMITATIONS: It is recommended that biochemical and/or serological tests be performed on colonies from pure culture for complete identification. Colonies tested should be 24-72 hours old. Some indole positive species may give a false positive result for L-proline aminopeptidase activity. It is important to note that *C. difficile* isolates should be indole negative and L-proline aminopeptidase positive. Further biochemical characterization and serological grouping may be necessary for specific identification. Mixed cultures can lead to false positives so pure cultures are needed for accurate results. Additionally, care must be taken so as not to pick up agar, which can lead to false positives.

PROCEDURE: Use a culture that is between 24 to 72 hours old to inoculate the Identicult- PRO Test card.

1. Apply 3-4 drops of Reagent #1 (Buffer) (provided) to the circle on the IDENTICULT-PRO test card. **Do not oversaturate.**
2. Touch the tops of 3-5 suspect colonies with the tip of a wooden applicator, swab, loop or needle and smear the growth onto the center of the circle/strip. Plated growth need not to be a pure culture provided morphologically similar, isolated colonies are selected. A single colony may be tested if growth is not well isolated or is sparse; however, the color reaction is stronger when a large inoculum is used.
3. Incubate the inoculated card at room temp. for 2-5 minutes.
4. Apply 2 drops of Reagent #2 to the circle. Wait at least 1 but not more than 2 minutes for color to develop.

INTERPRETATION:

Positive: The appearance of a dark pink to red color is positive. Indole reactions of organisms grown on any media containing tryptophane (e.g. blood agar) may interfere but will still produce predictable reactions. From such media, positive PRO reactions will range from dark pink or red (PRO+/indol-) to purple (PRO+/indole+).

Negative: Green or turquoise (indole+), and yellow (indol-) are both PRO negative.

QUALITY CONTROL:

Microorganisms Used	(ATCC #):	Expected Results:
<i>Clostridium difficile</i>	(9689)	+
<i>Clostridium perfringens</i>	(13124)	-

USER QUALITY CONTROL: Each lot should be tested with known positive and negative organisms. Some suggested strains are *C. difficile* ATCC 9689 (positive) and *C. perfringens* ATCC 13124 (negative). Positive PRO and Indole may be shown by testing with *C. sordelli*. Dispose of all used material in a manner appropriate for hazardous material.

BIBLIOGRAPHY:

- (1) Manual of Clinical Microbiology, Fifth Edition, ASM, Washington, D.C.
- (2) Wadsworth Anaerobic Bacteriology Manual, 5th Edition, 1993, Glucosidase tests, page 152.



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