

## Enzyme Confirmation Tests

Synonyms: Enzyme Test Cards

### Product Description

These tests utilize a specific substrate which, when hydrolyzed by a specific enzyme of the target organism (during peptide hydrolysis), produces a blue/white fluorescence, or purple/blue color upon the addition of Reagent B (color developer) when applicable.

### Components

(P/N: See Table 1. Sufficient for 48 tests)  
 1 Bottle of Reagent A (Buffer)  
 12 Test Cards (4 Test Spots on Each Card)  
 1 Bottle of Reagent B (Color Developer if Provided)  
 1 Product Insert

NOT PROVIDED: Swabs, inoculating needles, wooden applicator sticks, positive and negative control microorganisms, long wave UV Light.

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices. Care should be taken against the hazards of viable bacteria. Do not touch the filter membrane before, or after use. Sterilize cards before discarding.

### Preparation Instructions

Prep Instructions

### Storage/Stability

All materials can be stored at room temperature (away from direct sun light). Refrigeration storage does not harm the test.

Do not use the test card after the expiration date has passed, or if the filter is no longer white, which could make the results invalid.

Over 500 isolates were tested with these test cards, with a correlation of greater than 99%, when compared to traditional biochemical testing.

USER QUALITY CONTROL: When using the Test, each time it should be checked with positive and negative controls, using known stock strains of target organism, and another strain of bacteria that is not the target organism. Ideally cultured bacteria should be no more than 18 hours old and grown on an agar plate specified in Table 1, otherwise effective bacterial enzyme may not be present.

### Procedure

It is recommended to use a culture incubated from an agar plate of the types specified in Table 1. Other types of growth media may not allow the target organism to produce the target enzyme to be detected by the test card. Ideally the growth is between **14 to 18 hours** old before inoculating the Test Card. *After 24 hours of growth most bacteria go into a stationary phase where reduced levels of enzymes are present which may affect the results.* If a test of negative result is done after 24 hours of growth and a concern remains, repeat the culture and test within 14 to 18 hours. (Note: The incubation temperature should be 44°C for fecal coliform, and 38.5°C for all other types of bacteria.)

1. Select one test spot as your Control Spot, and other test spots as your Sample Spot or positive control. Add 1 drop of Reagent A to the filter membrane area of the Control Spot and to the filter membrane area of each Sample Spot.
2. Select colonies that morphologically resemble the target organism from the first growth plate and touch the tops of 1-2 colonies with a loop, inoculating needle, swab, or wooden applicator. Smear the colonies onto the filter membrane area of the Sample Spot ONLY.



**Set aside the inoculated card at room temperature for 5-10 minutes.**

**A) UV detection method (see Table 1)**

- Look at the test card under a long wave (~360nm) UV light. A positive test will be indicated by a **fluorescence light** (see Table 1) around the smeared colonies in the Sample Spot over a blue background. The test is negative if no fluorescence colonies appear in the Sample Spot (which will look like the Control Spot). A positive test is indicative of the target organism. The fluorescence of a positive result should remain on the Sample Spot for more than 24 hours. Stop here.

**B) Color detection method (see Table 1)**

- Add 1 drop of Reagent B to the filter membrane area of the Sample Spot and to the filter membrane area of each Sample Spot.
- A **purple or blue** (as stated in Table 1) color will immediately form on and around the deposited colonies on the Sample Spot in the presence of the hydrolyzed substrate, indicating a **Positive** test of the target organism. The test is negative if no purple or blue color appears in the Sample Spot. The test result must be read within 1 minute after the addition of Reagent B, otherwise the test result may be invalid. If purple or blue color shows on the Control Spot then re-test.

**LIMITATIONS OF THE TEST:**

Occasionally some species of other microorganisms *may* produce small amounts of enzyme, which can produce a positive test from culture. This is extremely rare.

**Technical Alert for Quality Control: The test detects bacterial enzymes, which may dissolve in liquids. Serial dilutions of bacteria may not give a positive reaction.**

**Enzyme Confirmation Test for Bacteria**

| Part Number | Target Organism              | Reagent B | Inspection Method | Shown Color if Positive | Suitable Growth Agar Media |    |   |     |      |    |     |    |    |    |
|-------------|------------------------------|-----------|-------------------|-------------------------|----------------------------|----|---|-----|------|----|-----|----|----|----|
|             |                              |           |                   |                         | B                          | M  | E | Sal | CSal | EC | 110 | MS | LT | CH |
| 75444       | <i>E. coli</i>               | No        | UV Light          | Blue/White Fluorescence | X                          | X* |   |     |      | X  |     |    | X  |    |
| 77643       | Total Coliform               | No        | UV Light          | Blue/White Fluorescence | X                          | X* |   |     |      |    |     |    | X  |    |
| 40926       | Fecal Coliform               | No        | UV Light          | Blue/White Fluorescence | X                          | X* |   |     |      |    |     |    | X  |    |
| 55283       | <i>Salmonella</i>            | No        | UV Light          | Yellow Fluorescence     | X*                         |    |   | X   | X    |    |     |    |    |    |
| 56305       | <i>Enterococcus</i>          | Yes       | Visual            | Purple                  | X*                         |    | X |     |      |    |     |    |    |    |
| 74203       | Gram+/Gram– Differentiation  | Yes       | Visual            | Purple (indicate Gram–) | X                          | X* |   |     |      |    |     |    |    |    |
| 92598       | <i>Neisseria gonorrhea</i>   | Yes       | Visual            | Blue                    |                            |    |   |     |      |    |     |    |    | X* |
| 77701       | Group A <i>Streptococcus</i> | Yes       | Visual            | Purple                  | X*                         |    |   |     |      |    |     |    |    |    |
| 80031       | <i>Staphylococcus aureus</i> | Yes       | Visual            | Purple                  | X                          |    |   |     |      |    | X   | X* |    |    |

**Table 1**

\* recommended media



#### Index List of Media:

- B = Blood Agar (inclus. Columbia Agar, TSA II with blood, e.g. Cat. No. 70133 and Cat. No. 27688)
- M = MacConkey Agar (e.g. Cat. No. 70143)
- E = Enterococcus Selective Agar (e.g. Cat. No. 45183)
- Sal = Salmonella agar (W/brilliant green) (e.g. Cat. No. 70134)
- CSal = Chromogenic Salmonella agar (e.g. Cat. No. 05538)
- EC = E. coli agar (eg. ECD Agar Cat. No. 44655?)
- 110 = Staphylococcus Agar No. 110 (e.g. Cat. No. 70193)
- MS = Mannitol Salt Phenol Red Agar (Cat. No. 63567)
- LT = Lauryl Tryptose agar (e.g Cat. No. 17349 + Agar)
- CH = Chocolate Agar (e.g. Cat. No. 70133 or Chocolate II Agar)

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