



COSMO BIO CO., LTD.

Inspiration for Life Science

(For research use only)

For guaiacol formation

Va-YSG Medium (2mL) 100 tubes [Catalog number: 08901]

For detection of acidophilic-thermotolerant bacteria

Guaiacol Detection Kit 100 tests [Catalog number: 08921]

Contents

Va-YSG Medium (2mL) 100 tubes(Catalog number: 08901)

Vanillic acid added YSG medium for guaiacol formation 2 mL x 100 tubes

Guaiacol Detection Kit 100 tests(Catalog number: 08921)

Reagent 1: 50mM Potassium hydrogen phthalate Buffer 60 mL x 2 bottles

Reagent 2: 1.3 % hydrogen peroxide solution 2.5 mL x 1 tube

Reagent 3: Peroxidase-Phosphoric Acid Buffer 2.5 mL x 1 tube

Positive Control: Guaiachole (1050 ppm) 2.5 mL x 1 tube

Method

(1) Take with single inoculation loop, 10 μ L, of young colony that has been isolated on YSG agar plate and that has not formed spores. Suspend the colony in a tube of “Va-YSG medium (2mL), ” ($\geq 1 \times 10^8$ cfu/mL).

(2) Incubate at $45 \pm 1^\circ\text{C}$ for 3 hours.

(3) Add 1.0 mL of Reagent 1 of “Guaiacol Detection Kit”.

(4) Add 20 μ L of Reagent 2 of “Guaiacol Detection Kit.

(5) Add 20 μ L of Reagent 3 of “Guaiacol Detection Kit.

(6) Mix well.

(7) Let it stand for 5-10 minutes.

(8) Check the color change of the solution.

*Read the result within 10 minutes

***Blank (Negative Control)**

Use “Va-YSG medium (2mL)” that has not been inoculated and follow steps (2)-(8).

***Positive Control**

Mix well 2 mL of purified water and 100 μ L of Positive Control. Follow steps (3)-(8).



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Interpretation of Results

Darker brown than blank = Positive

*If the color change is not significant and difficult to determine, retest with 24 hours incubation instead of 3 hours, step(2)

Same color as blank = Negative

Verify the test results by checking the reading of positive control; positive control result should have significantly darker brown than blank.

Storage

Store in dark and refrigerated (2-10 °C)

[Reference]

- 1) Niwa,M., A.Kawamoto, Development of a rapid detection method of *A. acidoterrestris*, hazardous bacteria to acidic beverage, *Fruit Processing*, 13,102-107(2003)
- 2) Niwa,M.(A course for “ Taxonomy and identification of microorganisms “ 1, Taxonomy and quality of new thermostable acidophile bacteria.) Technical data of soft drinks 2001,(1) 9-26(2001)
- 3) Niwa,M. et.al. (Development of a rapid detection of *A. acidoterrestris*, hazardous bacteria to acidic beverage) Report of Association for Fruit Juice (531) 23-30
- 4) Niwa, M. A. Kuriyama, *A. acidoterrestris* RAPID DETECTION KIT, *Fruit Processing*, 13,328-331(2003)
- 5) Niwa,M. et.al., Report of Association for Fruit Juice (541)(Sep. 2003), (Proposal for direct detection of of *A. acidoterrestris* using peroxidase method)

manufactured by

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