



ONPG Discs

DD008

ONPG Discs are used for the rapid detection of b-galactosidase activity in microorganisms, specially to identify late lactose fermenters quickly.

Directions

Place one ONPG disc in a sterile test tube. Add 0.1 ml of sterile 0.85% w/v sodium chloride solution (physiological saline). Pick up the colony under test with a sterile loop and emulsify it in physiological saline in the tube containing the disc. Incubate at 35-37°C. To detect active lactose fermenters observe the tube at an interval of one hour, for upto 6 hours. To detect late lactose fermenters, incubate the tubes for upto 24 hours.

Precautions

The reaction speed depends upon the size of inoculum. Use known positive and negative beta-galactosidase producing organisms to monitor the disc reactions.

Principle And Interpretation

ONPG (Ortho-nitrophenyl beta-D-galactopyranoside) is a synthetic colourless compound (galactoside) structurally similar to lactose (1).

beta-galactosidase cleaves ONPG to galactose and o-nitrophenyl, a yellow compound. The ONPG test is specially useful in the rapid identification of cryptic lactose fermenters (late fermenters). Since members of family *Enterobacteriaceae* are routinely grouped according to their lactose fermenting ability the ONPG test is significant here.

ONPG discs are sterile filter paper discs impregnated with ONPG. ONPG is similar in structure to lactose. The presence of two enzymes is required to demonstrate lactose fermentation in a conventional test. The first enzyme permease, facilitates the entry of lactose molecules into the bacterial cell while the second enzyme, beta-galactosidase, hydrolyzes the lactose to yield glucose and galactose. True non-lactose fermenters lack both enzymes; however some organisms lack permease but possess beta-galactosidase. These organisms are late lactose fermenters.

Quality Control

Appearance

Filter paper discs of 6 mm diameter bearing letters "On" in continuous printing style.

Cultural response

ONPG reaction observed in 0.85% sodium chloride solution of following culture containing ONPG (DD008) disc after an incubation of upto 4 hours at 35-37°C.

| Organism | ONPG |
|---|-------------------------------------|
| <i>Citrobacter freundii</i> ATCC 8090 | Positive reaction: yellow colour |
| <i>Enterobacter aerogenes</i> ATCC 13048 | Positive reaction: yellow colour |
| <i>Escherichia coli</i> ATCC 25922 | Positive reaction: yellow colour |
| <i>Salmonella Choleraesuis</i> ATCC 12011 | Positive reaction: yellow colour |

| | |
|--|---|
| <i>Proteus vulgaris</i> ATCC 13315 | Negative reaction: no colour change |
| <i>Salmonella Typhimurium</i> ATCC 14028 | Negative reaction: no colour change |

Storage and Shelf Life

Store at 2-8°C. Use before expiry date on the label.

Reference

1.Lowe G.H., 1962., J. Med. Lab. Technol., 19:21

Revision : 1 / 2011



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