

Technical Data

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 posses 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
Citrobacter freundii ATCC	Positive
8090	reaction:
	yellow colour
Enterobacter aerogenes	Positive
ATCC 13048	reaction:
	yellow colour
Escherichia coli ATCC	Positive
25922	reaction:
	yellow colour
Salmonella Choleraesuis	Positive
ATCC 12011	reaction:
	yellow colour

Proteus vulgaris ATCC	Ν
13315	re
	co
Salmonella Typhimurium	Ν
ATCC 14028	re

Negative reaction: no colour change 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

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