

49940 ONPG Disks (2-Nitrophenyl β -D-galactopyranoside Disks, β -Galactosidase Test Disks)

ONPG Disks are used to detect the presence of β -galactosidase, an enzyme found in lactose-fermenting organisms. Lactose utilization depends upon two enzymes: β -galactoside permease (not present in late lactose fermenters), which catalyzes transport of lactose into the cell, and β -galactosidase, which breaks down lactose into galactose and glucose. β -Galactosidase is not lactose specific and can act on simple galactosides including the ONPG (o-nitrophenyl- β -D-galactopyranose) substrate. ONPG hydrolysis results in the release of galactose, and the yellow chromogenic compound, o-nitrophenol. The test substrate, ONPG, does not depend on an induced or constitutive permease enzyme to enter the cell, therefore reactions are rapid and occur within a 24-hour period even for late lactose fermenters.

To group enterobacteriaceae the ability of fermenting lactose is routinely used.

Composition:

(1 package contains 50 disks)

Sterile filter paper disks (diameter 6mm) impregnated with o-nitrophenyl- β -D-galactopyranose

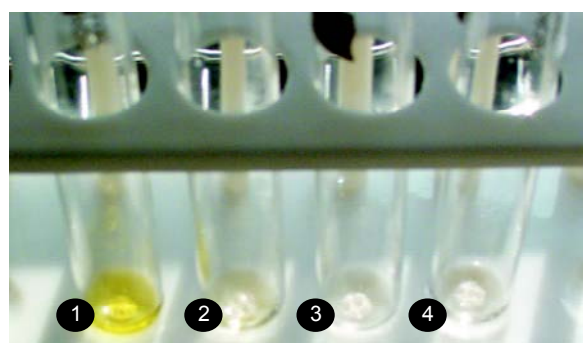
Directions:

Place one ONPG disk into 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 the tube containing the disk and physiological saline. Incubate at 35°C. To detect active lactose fermenters observe the tube at an interval of one hour, for up to 6 hours. To detect the late lactose fermenters, incubate the negative tubes for up to 24 hours.

Quality control:

Cultural characteristics in 0.85% (w/v) sodium chloride solution with an ONPG Disk after 4 hours at 35°C.

Test Organisms (ATCC)	ONPG Hydrolysis
<i>Citrobacter freundii</i> (8090)	+
<i>Enterobacter aerogenes</i> (13048)	+
<i>Escherichia coli</i> (25922)	+
<i>Proteus vulgaris</i> (8427)	-
<i>Salmonella serotype Arizonae</i> (13314)	+
<i>Salmonella serotype Typhimurium</i> (14028)	-



1. Positive Colony
2. Negative Colony
3. Negative Colony
4. Negative Control

References:

1. W.L. Gaby, C. Hadley, J. Bact., 74, 356 (1957)
2. J. Sanbrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual 2nd ed., Cold Spring Harbor, NY (1989)
3. S.R. Maloy, J.E. Conran (Jr.), D. Freifelder, Microbial Genetics 2nd ed. Jones and Bartlett Boston, MA (1994)
4. V.E. Becker, H.J. Evans, The influence of monovalent cations and hydrostatic pressure on β -galactosidase activity., Biochim. Biophys. Acta, 191, 95 (1969)
5. M.C. Neville, G.N. Ling, Synergistic activation of β -galactosidase by Na⁺ and Cs⁺., Arch. Biochem. Biophys., 118, 596, (1967)
6. J. Lederberg, The β -galactosidase of *Escherichia coli*, strain K-12., J. Bact., 60, 381 (1950)