



oNPG DISKS

- For in vitro use only -

Catalogue No. DO55

Our 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, 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. 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. oNPG Disks can be used to:

1. Differentiate late lactose fermenters: *Citrobacter* spp. (+), *Salmonella bongori* (+), *Salmonella enterica* subsp. *arizonae* (+), and *Salmonella enterica* subsp. *diazona* (+) from non-lactose fermenters: most other *Salmonella* species (-)
2. Differentiate *Burkholderia cepacia* (+) and *Stenotrophomonas maltophilia* (+) from *Pseudomonas* species (-)
3. Differentiate *Neisseria lactamica* (+) from other *Neisseria* species (-)
4. Differentiate *Shigella sonnei* (+) from other *Shigella* species (-)

Recommended Procedure

1. Inoculate 0.2 mL of 0.85% saline (pH 7.2) with an inoculum of the organism to be tested obtained from a lactose-containing medium such as KIA or TSI.
2. Aseptically, add an oNPG disk to the suspension.

3. Incubate at 35°C.
4. Check after 20 minutes, and hourly for six hours.
5. If there is still no color change, re-incubate for up to 24 hours.

Interpretation of Results

Positive: Yellow color

Negative: No color change

- Do not use disks if they are yellow or discolored
- The inoculum must be recovered from a lactose containing medium given the inducible nature of β -galactosidase
- The heavier the inoculum the higher the concentration of enzyme thereby increasing the speed of a positive reaction

Quality Control

<u>Organism</u>	<u>Expected Results</u>	
<i>Escherichia coli</i> ATCC 25922	+ve	Yellow
<i>Proteus mirabilis</i> ATCC 12453	-ve	No color change

Storage and Shelf Life

oNPG Disks should be stored at -20°C and protected from light. At this temperature they have a shelf life of 32 weeks from the date of manufacture.

References

1. Lowe, GH. J Med Lab Technol 1962; 19:21-6.
2. Forbes BA, Sahm DF Weissfeld AS. Bailey and Scott's diagnostic microbiology. 10th ed. St. Louis: Mosby, 1998.
3. MacFaddin, JF. Biochemical Tests for the Identification of Medical Bacteria, 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

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