ONPG /Disks

355-3822

DEFINITION

Certain Enterobacteria possess:

- the enzyme necessary for the use of lactose by the bacteria (β-galactoside-permease);
- as well as the enzyme splitting the lactose molecule into glucose and galactose (β-galactosidase).

If the first of these two activities is deficient or absent, a potentially "lactose-positive" bacteria - because it possesses a β-galactosidase - would be unable to express this character and would appear "lactose-negative". Acidification of a lactose medium will manifest only later, if a reverse mutant appears, or perhaps not occur at all.

Reaction:

Orthonitrophenyl-β-D-Galactopyranoside (ONPG) in solution is colourless.
The molecule is split, like lactose, by β-galactosidase, releasing the orthonitrophenol, yellow in solution.
This reaction is carried out in buffered solution.
The disks are impregnated with ONPG + buffer.

PRESENTATION

Set of 50 disks **code 355-3822**

STORAGE

- Ready to use: + 2°C to 8°C.
- Expiration date and batch number are shown on the package.
- In the event of doubts over the validity, check using a positive strain.

PROTOCOL

Bacteria, collected from agar medium, are placed in a thick (milky) suspension in 0,5 ml of distilled water in a small tube (e.g. Kahn tube). The more bacteria, the more enzymes, and therefore the reaction will be all the more rapid.

Place a disk in this suspension and incubate in a water-bath at 37°C.

READING AND INTERPRETATION

Observe after 1 hour of incubation.
The vast majority of positive reactions (yellow colour) occur in 15 to 30 minutes.

Results obtained with aerobic or anaerobic Gram-negative bacilli:

	ONPG positive release of orthonitrophénol = yellow solution	**ONPG negative = colorless solution**
ENTERO-BACTERIA	*E. coli* (the majority of strains of *Alkalescens dispar* biotype are ONPG -) *Shigella sonnei* biotype xylose – *Shigella dysenteriae* 1 *Shigella dysenteriae* 6 *Shigella boydii* 9 *Salmonella* sub-species III (*S. arizona*) *Salmonella* sub-species II (a) *Citrobacter* *Levinea malonatica* *Levinea amalonatica** *Klebsiella* (except *K. rhinoscleromatis*) *Enterobacter Hafnia** *Serratia marcescens** *Serratia liquefaciens* *Serratia plymutica* *Yersinia**	Other *Shigella* *Salmonella* sub-species Iet IV *Edwardsiella* *Proteus* *Providencia*
VIBRIO-NACEAE	*Vibrio cholerae** *Vibrio anguillarum** *Plesiomonas*	*Vibrio parahaemolyticus* *Vibrio alginolyticus*
AEROMONAD-ACEAE	*Aeromonas hydrophila** *Aeromonas sobria*	

*** hydrolyses ONPG due to an enzyme other than β-galactosidase in the strictest sense of the term, i.e. can be induced by IPTG.**

PRECAUTIONS

Respect of Good Laboratory Practice.

Bio-Rad, 3 boulevard Raymond Poincaré • 92430 Marnes-la-Coquette
Tel. : +33 (0)1 47 95 60 00 • Fax : +33 (0)1 47 41 91 33
www.foodscience.bio-rad.fr

BIBLIOGRAPHY

- **RICHARD Cl. (1978):** Annales Biologiques Clin., **36**: 407-424.
- **LE MINOR L., COYNAULT C., GUISON N. (1977):** Annales de l'Institut Pasteur, **128 B**: 35-43.
- **POINDRON P., BONLOUIS C. (1974):** Méd. Mal. Infect., **4**: 23-28.
- **LE MINOR L., BEN HAMIDA F. (1962)**: Annales de l'Institut Pasteur, **102**: 267-277.
- **MOLLARET H., LE MINOR L. (1962):** Annales de l'Institut Pasteur, **102**: 649-652.

Bio-Rad, 3 boulevard Raymond Poincaré • 92430 Marnes-la-Coquette
Tel. : +33 (0)1 47 95 60 00 • Fax : +33 (0)1 47 41 91 33
www.foodscience.bio-rad.fr