

DESOXYCHOLATE LACTOSE AGAR

ENUMERATION OF COLIFORMS

1 INTENDED USE

Desoxycholate Lactose Agar is a selective medium for the enumeration of coliform bacteria in water, milk, dairy and other food products. The medium is also used for the differentiation and isolation of enterobacteria from samples of animal origin.

2 HISTORY

In 1935, Leifson developed the formulation of desoxycholate lactose as a differentiation medium for enterobacteria. In contrast to prior formulas using ingredients of unknown or variable composition, this medium contained well defined chemical substances, such as desoxycholate and sodium citrate, used as inhibitors. The degree of inhibition was thus well controlled. The original formulation of Leifson was slightly modified in terms of the use of better defined peptones, as well as by non-negligible adjustments of the inhibitory substances.

3 PRINCIPLES

Gram-positive bacteria are inhibited by desoxycholate, although sodium citrate is also an effective inhibitor.

The differentiation of enterobacteria is based on their capacity to ferment lactose :

- Lactose-positive strains produce acid which leads to the formation of red colonies in the presence of neutral red.
- Lactose-negative bacteria yield colorless colonies (*Salmonella* or *Shigella*)

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Peptic digest of Meat.....	10,00 g
- Lactose.....	10,00 g
- Sodium desoxycholate	0,50 g
- Sodium chloride	5,00 g
- Sodium citrate	2,00 g
- Neutral red.....	0,03 g
- Bacteriological agar.....	15,00 g

pH of the ready-to-use media at 25 °C : 7,1 ± 0,2.

5 PREPARATION

- Dissolve 42,5 g of dehydrated media (BK065) into 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Do not autoclave.
- Cool and maintain the media in a molten state at 44-47 °C.

✓ **Reconstitution :**
42,5 g/L

✓ **Sterilization :**
Do not autoclave

6 INSTRUCTIONS FOR USE

- Transfer 1 mL of the product to analyze and its serial tenfold dilutions into sterile Petri plates.
- Pour in roughly 12 mL of molten media.
- Homogenize by swirling and let solidify on a cold, flat surface.
- Overlay by pouring in an additional 4 mL of medium, so that a second layer is formed. Let solidify.
- Incubate at 30 ± 1 °C or at 37 ± 1 °C for 18 to 24 heures according to the analytical protocol being followed.

✓ **Inoculation :**
1 mL double layer

✓ **Incubation :**
18 to 24 h at 30 or 37 °C

7 RESULTS

Characteristic colonies are those which are red and with a diameter equal to or greater than 0.5 mm after 24 hours of incubation.

Lactose-negative *Enterobacteriaceae* give rise to colorless colonies.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : pinkish powder, free-flowing and homogeneous.

Prepared media : pinkish-red agar.

Typical culture response after 24 hours of incubation at 30 °C :

Microorganisms	Growth (Productivity Ratio : P_R)	Characteristic colonies
<i>Escherichia coli</i> WDCM 00013	$P_R \geq 50$ %	Red-pink colonies w/ precipitated bile
<i>Escherichia coli</i> WDCM 00012	$P_R \geq 50$ %	Red-pink colonies w/ precipitated bile
<i>Citrobacter freundii</i> CIP 57.32T	$P_R \geq 50$ %	Red-pink colonies w/ precipitated bile
<i>Enterococcus faecalis</i> WDCM 00087	Inhibited, score 0	-
<i>Staphylococcus aureus</i> WDCM 00034	Inhibited, score 0	-

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.

The expiration date is indicated on the label.

Prepared media (*) : Use on the day of preparation.

(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :

500 g bottle BK065HA

11 BIBLIOGRAPHY

Leifson, E.. 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. Journal of Pathology and Bacteriology, **40** : 581-599.

Journal Officiel du 17 février 1985. Méthodes d'analyses des laits pasteurisés (Arrêté du 3 janvier 1985).

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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Desoxycholate Lactose Agar

Enumeration of coliforms

Results :

Growth obtained after 24 hours of incubation at 37°C.



Escherichia coli

Characteristic colony :
Red-pink color surrounded by halo of
precipitated bilee.