TECHNICAL DATA SHEET

DICHLORAN-GLYCEROL (DG 18) AGAR

ENUMERATION OF YEASTS & MOLDS

1 INTENDED USE

Dichloran-glycerol (DG-18) agar is recommended for the enumeration of yeasts and molds that develop in products with a low water activity (a_w less than 0.95). The media finds particular applications for the enumeration and isolation of xerophilic molds which may be found in dehydrated or extremely dry products, such as heavily sugared or salted foods, dried fruits, cereals, cakes and cookies, flour and meat or fish based dehydrated products. The media favors a controlled growth regarding the size and breadth of mold mycelia and yeast colonies, allowing easier and more accurate counts.

The typical composition of the media corresponds to that defined in the standards NF V08-036 & NF ISO 21527-2.

2 HISTORY

DG18 agar is derived from the formula described by Hocking and Pitt, in 1980 for the enumeration of molds that develop in products with low water content. In 2001, Deak et al. demonstrated, using a comparative study, that cultures of *Brettanomyces anomalus*, *Cryptococcus albidus*, and *Rhodotorula mucilaginosa* were significantly reduced or even inhibited on this medium. Conversely, xerophilic yeasts such as *Zygosaccharomyces rouxxi* were easily cultivated. These authors concluded that DG18 agar was particularly well adapted to products with low water content and consequently, was not considered to have universal applications for the enumeration of a large spectrum of contaminating yeasts and molds in all food products.

3 PRINCIPLES

Tryptone and glucose assure the growth of the yeasts and molds.

The concentration of glycerol at 18% results in a diminution of the water content from 0.999 to 0.955.

Dichloran inhibits the invasion of molds and reduces the size of other colonies.

The presence of Chloramphenicol, a heat resistant antibiotic, reinforces the selectivity of the medium against the majority of bacterial contaminants.

4 TYPICAL COMPOSITION

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

For 1 liter of complete media :

- Tryptone ..... 5,0 g
- Glucose ..... 10,0 g
- Monopotassium phosphate ..... 1,0 g
- Magnesium sulfate, H_2O ..... 0,5 g
- Dichloran (dichloro-2,6-nitro-4-aniline)..... 2,0 mg
- Chloramphenicol ..... 0,1 g
- Glycerol ..... 220,0 g
- Bacteriological agar..... 13,0 g

pH of the ready-to-use media at 25 °C : 5,6 ± 0,2.

For 29,6 g of dehydrated base BK170

- Tryptone ..... 5,0 g
- Glucose ..... 10,0 g
- Monopotassium phosphate ..... 1,0 g
- Magnesium sulfate, H_2O ..... 0,5 g
- Dichloran (dichloro-2,6-nitro-4-aniline) ..... 2,0 mg
- Chloramphenicol ..... 0,1 g
- Bacteriological agar ..... 13,0 g

Glycerol not included; must be added extemporaneously

For 1 liter of ready-to-melt (BM109)

- Tryptone ..... 5,0 g
- Glucose ..... 10,0 g
- Monopotassium phosphate ..... 1,0 g
- Magnesium sulfate, H2O ..... 0,5 g
- Dichloran (dichloro-2,6-nitro-4-aniline) ..... 2,0 mg
- Chloramphenicol ..... 0,1 g
- **Glycerol ..... 220,0 g**
- Bacteriological agar ..... 13,0 g

5 PREPARATION

Dehydrated media preparation :

- Dissolve 29.6 g of dehydrated base media (BK170) in 1 liter of distilled or demineralized water.
- Add 220.0 g of glycerol .
- Slowly bring to a boil, stirring with constant agitation until complete dissolution.
- Dispense into tubes or vials,100 mL per container.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain at 44-47 °C.

✓ **Reconstitution :**
29,6 g/L
+ 220 g/L of glycerol

✓ **Sterilization :**
15 min at 121 °C

6 INSTRUCTIONS FOR USE

Pour plate inoculation (NF V08-036)

- Transfer 1 mL of the product to analyze and its serial tenfold dilutions to sterile Petri dishes.
- Pour roughly 15 mL of molten medium to each plate.
- Homogenize by swirling. Let solidify on a cool surface.
- Incubate at 25 ± 1 °C for 5 days.

✓ **Inoculation :**
1 mL in depth

✓ **Incubation :**
5 days at 25 ± 1 °C

Surface inoculation (NF ISO 21527-2)

- Pour the media into sterile Petri plates.
- Dry the plates in an incubator with the covers partially removed.
- Transfer 0.1 mL of the product to analyze and its serial tenfold dilutions into the surface of plates.
- Inoculate the sample by streaking on the surface.
- Incubate plates, lids uppermost, at 25 ± 1 °C for 5 to 7 days.

✓ **Inoculation :**
0.1 mL on surface

✓ **Incubation :**
5 to 7 days at 25 ± 1 °C

7 RESULTS

Count those plates containing less than 150 colony forming or mycelial forming units.

8 QUALITY CONTROL

Dehydrated media : cream powder, free-flowing and homogeneous.
Prepared media : amber agar.

Typical culture response after 5 days of incubation at 25°C (NF EN ISO 11133) :

Microorganisms		Growth (Productivity Ratio : P_R)
Saccharomyces cerevisiae	WDCM 00058	$P_R \geq 50\%$
Wallemia sebi	WDCM 00182	$P_R \geq 50\%$
Escherichia coli	WDCM 00013	Inhibited
Bacillus subtilis ssp. *spizizenii*	WDCM 00003	Inhibited

9 STORAGE / SHELF LIFE

Dehydrated base media (without glycerol) : 2-20 °C.
Complete ready-to-melt media (with glycerol), in vials : 2-8 °C.
The expiration dates are indicated on the labels.

Prepared media in vials (*): 90 days at 2-8 °C.
Complete, prepared media in plates (*): 8 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated base media (without glycerol) :
500 g bottle BK170HA

Complete, ready-to-melt media (with glycerol) :
10 vials x 100 mL BM10908

11 BIBLIOGRAPHY

Hocking, A.D., and Pitt, J.I. 1980. Dichloran-glycerol medium for enumeration of xerophilic fungi from low moisture foods. App. Environ. Microbiol.,39: 488-492.

Deak, T., Chen, J., Golden, D.A., Tapia, M.S., Tornai-Lehoczki, J., Viljoen, B. C., Wyder, M.T., and Beuchat, L.R. 2001. Comparison of dichloran 18% glycerol (DG18) agar with general purpuse mycological media for enumerating food spoilage yeasts. Inter. Jour. of Food Microb. 67: 49-53.

NF V 08-036. Mai 2003. Microbiologie des aliments. Méthode horizontale pour le dénombrement des levures et moisissures se développant sur un milieu à faible a_w.

NF ISO 21527-2. Novembre 2008. Microbiologie des Aliments. Méthode horizontale pour le dénombrement des levures et des moisissures. Partie 2 : Technique par comptage des colonies dans les produits à activité d'eau inférieure ou égale à 0,95.

NF EN ISO 11133. Juillet 2014. Microbiologie des aliments, des aliments pour animaux et de l'eau. Préparation, production, stockage et essais de performance des milieux de culture.

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.

Document code : DG18_ENv9
Creation date : 04-2003
Updated : 03-2017
Origin of revision : storage temperature modification