

Dichloran Glycerol (DG-18) Agar Base (NCM0081)

Intended Use

Dichloran Glycerol 18% (DG18) Agar Base is a selective agar for the enumeration of viable osmophilic yeasts and xerophilic molds in products intended for human consumption or feeding of animals that have a water activity less than or equal to 0.95, by means of the colony count technique at $25 \pm 1^\circ\text{C}$, as described in ISO 21527-2:2008. Dichloran Glycerol 18% (DG18) Agar Base is not intended for use in the diagnosis of disease or other conditions in humans.

Description

A selective, agar for the enumeration of viable osmophilic yeasts and xerophilic molds in products intended for human consumption or feeding of animals that have a water activity less than or equal to 0.95 (dry fruits, cakes, jams, dried meat, salted fish, grains, cereals and cereal products, flours, nuts, spices and condiments, etc.), by means of the colony count technique at $25 \pm 1^\circ\text{C}$, as described in ISO 21527-2:2008.

The medium is based on the original formulation described by Hocking and Pitt (1980) and is widely used for food or animal feed products with a water activity of less than or equal to 0.95. This medium is not suitable for the examination of products with a water activity of greater than 0.95, Dichloran Rose Bengal Chloramphenicol Agar (NCM0082) should be used.

The reduction in water activity in this medium is achieved by the addition of glycerol at approximately 18% and this is very important as many yeast and molds require a low water activity to enhance growth and colony development. The medium also contains the antifungal agent Dichloran, which restricts the spreading of mucoraceous fungi and restricts the colony size of other genera making colony counting an easier task. Additional selectivity against bacterial growth is achieved by the incorporation of the heat-stable antibiotic Chloramphenicol. Glucose is incorporated as the fermentable carbohydrate source, with casein enzymatic digest providing the essential vitamins, minerals, amino acids, nitrogen and carbon. According to ISO 21527-2:2008 a specified quantity of sample or initial suspension is surface inoculated onto DG18 Agar plates. This medium conforms to the performance and formulation requirements of ISO 21527-2:2008. This medium conforms to the performance and formulation requirements of ISO 21527-2:2008.

Typical Formulation

Casein Enzymatic Digest	5.0 g/L
D-Glucose	10.0 g/L
Potassium Dihydrogen Phosphate	1.0 g/L
Magnesium Sulfate	0.5 g/L
Dichloran	0.002 g/L
Chloramphenicol	0.1 g/L
Agar	15.0 g/L

pH: 5.6 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 31.6 grams of medium and 220 grams of glycerol in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. DO NOT OVERHEAT
5. Cool to $45\text{--}50^\circ\text{C}$.



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Technical Specification Sheet



Test Procedure

For the enumeration of viable osmophilic yeasts and xerophilic moulds- Refer to ISO 21527-2:2008

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is beige, clear to slightly hazy.

Expected Cultural Response: Cultural response at $25 \pm 1^\circ\text{C}$ for up to 5 days of incubation.

<u>Microorganism</u>	<u>Approx. Inoculum (CFU)</u>	<u>Expected Results</u>
<i>Saccharomyces cerevisiae</i> WDCM 00058	50-200	>50% Recovery*
<i>Wallemia sebi</i> WDCM 00182	50-200	>50% Recovery*
<i>Aspergillus restrictus</i> WDCM 00183	50-200	>50% Recovery*
<i>Eurotium rubrum</i> WDCM 00184	50-200	>50% Recovery*
<i>Escherichia coli</i> WDCM 00012	$>10^4$	No growth
<i>Escherichia coli</i> WDCM 00013	$>10^4$	No growth
<i>Bacillus subtilis subsp. Spizizenii</i> WDCM 00003	$>10^4$	Inhibited

*Characteristic colonies/propagules according to each species

The organisms listed are the minimum that should be used for quality control testing.

Results

Fermentation of the carbohydrate source glucose, and water activity reduction from the glycerol addition, supports the growth of yeasts and molds.

The anti-fungal agent Dichloran inhibits spreading molds and restricts colony size, to improve enumeration and detection.

Additional selectivity is achieved by the Chloramphenicol, which allows inhibition of bacterial growth.

Expiration

The dehydrated medium should be discarded if it is not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Complete classification of yeast and molds is dependent upon microscopic observations of direct and/or slide culture preparations, along with biochemical and serological tests.
2. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage

Store dehydrated culture media at $2-30^\circ\text{C}$ away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.



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References

1. ISO 21527-2:2008 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and molds. Part 2: Colony count technique in products with water activity less than or equal to 0,95.
2. ISO 11133:2014+A1:2018 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.
3. Hocking, A.D., Pitt, J.I. (1980). Dichloran-glycerol based medium for the enumeration of xerophilic fungi from low moisture foods. *Appl. Environ. Microbiol.* **39**, 488-492.

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