IFU

Instructions for Use

DG-18 (DICHLORAN GLYCEROL) AGAR

Cat. no. W85	DG-18 Agar, 15x100mm Plate, 26ml	10 plates/bag

INTENDED USE

Hardy Diagnostics DG-18 Agar is recommended as a selective medium for the isolation and cultivation of xerophilic molds.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Hardy Diagnostics DG-18, or Dichloran Glycerol, Agar is based on a formulation by Hocking and Pitt and is recommended for the enumeration of xerophilic yeasts and molds from dried and semi-dried foods.[8] Examples include fruits and spices, confectionery products, cereals and nuts, and dried meat and fish. This medium is highly selective, has a high osmotic pressure, and allows for the enumeration of fungal growth. A parallel study using DG-18 Agar and DRBC, or Dichloran Rose Bengal Chloramphenicol, Agar showed that DG-18 Agar exhibited a greater recovery and more robust growth of two molds, *Aspergillus penicilloides* and *Wallemia sebi*, commonly found in dried foods.[8]

Hardy Diagnostics DG-18 Agar contains peptones which provide nitrogen, vitamins and amino acids required for microbial growth. Glucose provides an energy source. Phosphate acts as a buffering agent. The inorganic salt, magnesium sulfate, stimulates fungal growth and enhances sporulation. Dichloran inhibits the spreading of mucoraceous fungi and restricts the colony size of other genera commonly present in food samples. Chloramphenicol inhibits typical bacterial colonies present in environmental and food samples. Agar acts as the solidifying agent. Glycerol lowers the water activity (a_W) of the media from approximately 0.999 to 0.95 and provides an additional carbon source.

FORMULA

Ingredients per liter of deionized water:*

Glucose	10.0gm
Peptone	5.0gm
Potassium Dihydrogen Phosphate	1.0gm
Magnesium Sulfate	0.5gm
Chloramphenicol	0.1gm
Dichloran	0.002gm
Glycerol	220.0ml

Agar	15.0gm

Final pH 5.6 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Refer to the appropriate references for applications using DG-18 Agar for yeast and mold testing.[1,2,7-9,11]

1. Process the sample in a stomacher bag, adding 40gm of sample to 200ml of 0.1% peptone water (Cat. no. U201). Note: For powdered products, shake periodically for 30 minutes using 0.1% peptone water.

2. Dilute the sample 1:10 in 0.1% peptone water.

3. Plate 0.1ml of the prepared sample per plate.

4. Incubate plates at 15-30ºC. and examine for growth for up to 7 days.

INTERPRETATION OF RESULTS

Observe and record the number of yeasts and/or molds present and report as the number of xerophilic colonies per gram of food.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Complete classification of yeasts and molds is dependent upon microscopic examination of direct and/or slide culture preparations, in addition to biochemical and serological analysis.

Due to nutritional variation, some strains may grow poorly or fail to grow entirely on this medium.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, 0.1% peptone water (Cat. no. U201), incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Saccharomyces cerevisiae ATCC® 9763	A	1-7 days	15-30°C	Aerobic	Growth
Escherichia coli ATCC® 25922	A	1-7 days	15-30°C	Aerobic	Partial to complete inhibition
Bacillus subtilis ATCC® 6633	A	1-7 days	15-30°C	Aerobic	Partial to complete inhibition

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Dairy Products,* APHA, Washington, D.C.

2. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods,* APHA, Washington, D.C.

3. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

4. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm.

5. Beckers, H.J. et al. 1982. *Inter. Stand. Org. Document* ISO/TC34/SC9/N151.

6. Beuchat, L.R. and C.A. Hwang. 1996. Evaluation of Modified Dichloran 18% Glycerol (DG18) Agar for Enumerating Fungi in Wheat Flour: A Collaborative Study. *Int. J. Food Microbiol.;* 29:161-166.

7. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

8. Hocking, A.D. and J.I. Pitt. 1980. Dichloran-glycerol Medium for Enumeration of Xerophilic Fungi from Low Moisture Foods. *Appl. and Environ. Microbiol.;* 39:488-492.

9. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

10. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

11. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* AOAC, Arlington, VA. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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