IFU

Instructions for Use

CRITERION™ UNIVERSAL BEER AGAR

Cat. no. C9250	CRITERION™ Universal Beer Agar	108g
Cat. no. C9251	CRITERION™ Universal Beer Agar	500g
Cat. no. C9252	CRITERION™ Universal Beer Agar	2kg
Cat. no. C9253	CRITERION™ Universal Beer Agar	10kg

INTENDED USE

Hardy Diagnostics CRITERION™ Universal Beer Agar is recommended for the cultivation of microorganisms significant to the brewing industry.

Dehydrated culture media is a raw material not intended for use in the diagnosis of human disease. For implementation, this product requires additional processing and supplementation of ingredients before use.

SUMMARY

Universal Beer Agar, or UBA, was developed by Kozulis and Page to isolate and enumerate a wide variety of contaminating microorganisms commonly encountered by breweries in the beer manufacturing industry.[6] Hardy Diagnostics CRITERION™ Universal Beer Agar is based on this formulation and contains a wide variety of nutrients, including tomato juice solids, yeast extract, peptonized milk, dextrose, and salts. To complete the formulation, beer is added to the heated agar base in order to approximate the environmental conditions present in the brewery. This composition ensures recovery of many organism types, including *Lactobacillus*, *Pediococcus*, *Acetobacter*, *Zymomonas* spp. and wild yeast strains capable of surviving or multiplying in pitching yeast, wort and beer during processing. The alcohol and hop content of the medium impede the growth of transient microorganisms, while allowing growth of microbes specifically adapted to brewery conditions.[4,7] If desired, additional selective agents, such as cycloheximide, can be added to the medium to suppress the growth of yeast and fungi, and further enhance the isolation of bacterial contaminants. To enhance colony differentiation, bromocresol green and powdered chalk can also be added to the medium before autoclaving, and will produce zones of decolorization around *Pediococcus* and some *Lactobacillus* colonies.[4]

FORMULA*

Gram weight per liter:	54.0g/L
Dextrose	16.1g
Peptonized Milk	15.0g
Tomato Juice Solids	12.2g
Yeast Extract	6.1g

Phosphate Buffer	0.62g
Magnesium Sulfate	0.12g
Sodium Chloride	6.0mg
Ferrous Sulfate	6.0mg
Manganese Sulfate	6.0mg
Agar	12.0g

Final pH 6.3 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle that contains dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic and will clump when exposed to moisture and air. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. Dehydrated culture media should be discarded if clumped, if the media is not free-flowing or if the color has changed from its original slightly opalescent, medium to dark amber color.

Store the prepared culture media at 2-8ºC and do not remove the container desiccant, if applicable.

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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 54g of the dehydrated culture media in 750ml of distilled or deionized water. Stir to mix thoroughly.

2. Boil to dissolve completely. Do not overheat.

3. Add 250mL of beer, without degassing, to the hot medium and mix slowly but completely.

4. Sterilize media in the autoclave at 118ºC for 12 minutes.

Note: The shelf life of in-house prepared media from dehydrated culture media is dependent upon preparation methods, container quality, equipment, storage conditions, and batch testing criteria and must be validated by the end user. Refer to *USP Microbiological Best Laboratory Practices <1117>* for more information on validation procedures.[1]

PROCEDURE

Allow the medium to come to room temperature prior to use. Consult appropriate references for more information on the correct procedure for use.[1-10]

Direct Plating Method of Use:

1. Perform a four quadrant streak of the sample to obtain well-isolated colonies. Alternatively, the spread plate technique can be used to evenly distribute the sample over the surface of the agar. For enumeration, successive serial dilutions of the original sample can be utilized and spread onto the plate.

2. Plates are incubated at 28-30 degrees C., both aerobically to detect *Acetobacter* species and anaerobically to detect microaerophilic *Lactobacillus* and *Pediococcus* species, as well as anaerobic *Zymomonas* spp., for up to three days and examined daily for growth.

Dilution Method of Use:

1. Dilute samples with sterile saline or sterile water to achieve a final desired concentration of 100 CFU per plate.

2. Spread 0.1ml of the sample over the entire surface of the medium using a sterile spreading device or perform the pour plate method.[1] Note: It is recommended that three replicates of each inoculum be performed, along with suitable blanks and controls.

3. Incubate plates anaerobically at 28 degrees C. for up to 7 days and examine daily for growth.

Environmental Sampling Method of Use:

1. Aseptically collect a surface sample using an appropriate neutralizing diluent or Barney Miller Broth. Collect the sample by rubbing the swab over the sample area (approximately 50cm^2), reversing directions between strokes.

2. Repeat the collection procedure three more times. When sampling utensils such as knives or ladles, run the swab over the entire surface of the instrument three times as described above.

3. If using a neutralizing diluent, transfer the swab to an appropriate transport broth such as Barney Miller Broth if there is a delay to plate the sample. The sample can be refrigerated in Barney Miller Broth for up to 24 hours prior to plating.

4. Shake each broth tube vigorously (50 cycles of 15cm in 10 seconds) prior to plating. Plate 1.0ml and 0.1ml samples using the four quadrant streak or spread plate technique as mentioned above to obtain isolated colonies. Incubate plates at 35 degrees C. for 40-48 hours, then calculate the number of colonies from the 50cm^2 sample area.[10]

INTERPRETATION OF RESULTS

Examine plates for the presence of microbial growth, noting the types of colonies formed. Pick representative colonies for subculture and further identification. For environmental sampling, count the number of colonies from the 50cm^2 sample area.

LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

Accurate counting may be difficult with molds or spreading colonies.

Rare, fastidious microorganisms may not grow on selective media formulations.

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, incubators, tubes, bottles, petri dishes, other culture media (e.g. Barney Miller Broth), etc., 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	
Pediococcus damnosus industrial strain	B	40-48hr	15-30°C	Anaerobic	Growth; clearing of agar
Lactobacillus brevis industrial strain	B	40-48hr	15-30°C	Aerobic	Growth; clearing of agar
Saccharomyces cerevisiae industrial strain	B	40-48hr	15-30°C	Aerobic	Growth

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

USER QUALITY CONTROL

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ Universal Beer Agar powder should appear homogeneous, free-flowing, and beige in color. The prepared medium should appear clear with a slight precipitate, and light amber in color.

REFERENCES

1. American Society of Brewing Chemists. 1975. Report of Subcommittee on Microbiological Controls. Proc. *Am. Soc. Brew. Chem.*; 33:75.

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3. Barney, M.C., E.J. Kot and E. Chicoye. 1990. Culture Medium for Detection of Beer Spoilage Microorganisms. *U.S. patent 4,906,573*.

4. Boatwright, J. and Kirsop, B.H. 1976. Sucrose Agar: A Growth Medium for Spoilage Organisms. *Journal of Inst. Brewing*; 82:343-346.

5. Goldammer, T. 2000. *The Brewer's Handbook, The Complete Book to Brewing Beer*. Beer spoilage organisms; 19:1-14. KVP Publishers, Clifton, VA.

6. Kozulis, J.A., and H.E. Page. 1968. *A New Universal Beer Agar Medium for the Numeration of Wort and Beer Microorganisms*. American Society Brewing Chemists Proc. p. 52-58.

7. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance Medical Bacteria,* Vol. I, p. 819-820. Williams & Wilkins, Baltimore, MD.

8. Murphy, D.T., and L.T. Saletan. 1970. Use of Microbiological Media in the Brewery. *Tech. Q. Master Brew. Assoc. Am.*; 7:182-187.

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

10. Tiedman, W.D., Chairman. 1948. *Technic for the Bacteriological Examination of Food Utensils*. Committee Report. American Journal of Public Health Yearbook.

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

HDQA 2207B [D]