IFU

Instructions for Use

CRITERION™ WL DIFFERENTIAL MEDIUM AND WL NUTRIENT MEDIUM

Cat. no. C7290	CRITERION™ WL Differential Medium	164.2gm
Cat. no. C7291	CRITERION™ WL Differential Medium	500gm
Cat. no. C7292	CRITERION™ WL Differential Medium	2kg
Cat. no. C7293	CRITERION™ WL Differential Medium	10kg
Cat. no. C7294	CRITERION™ WL Differential Medium	50kg
Cat. no. C7300	CRITERION™ WL Nutrient Medium	164.2gm
Cat. no. C7301	CRITERION™ WL Nutrient Medium	500gm
Cat. no. C7302	CRITERION™ WL Nutrient Medium	2kg
Cat. no. C7303	CRITERION™ WL Nutrient Medium	10kg
Cat. no. C7304	CRITERION™ WL Nutrient Medium	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ WL Differential Medium is used for isolating bacteria encountered in brewing an industrial fermentation processes.

Hardy Diagnostics CRITERION™ WL Nutrient Medium is used for cultivating yeasts, molds, and bacteria encountered in brewing and industrial fermentation processes.

WL Nutrient Medium can support the growth of bacteria, but unless the yeast cell titer is small the bacteria may not be detected. Consequently, WL Differential Medium was created to inhibit the growth of yeasts without inhibiting the growth of bacteria present in beers.

SUMMARY

WL Nutrient Medium, also known as Wallerstein Laboratory Medium, was developed by Green and Gray while studying various fermentation processes.[4,5] WL Differential Medium and WL Nutrient Medium are used simultaneously as a set of three plates. One plate is prepared from WL Nutrient Medium and two plates from WL Differential Medium.[6] The WL Nutrient Medium plate is incubated aerobically to obtain a total count of mainly yeasts colonies. A second WL Differential Medium plate is incubated aerobically for growth and acetic acid bacteria including *Flavobacterium*, *Proteus* and thermophilic bacteria. A third WL Differential Medium plate is incubated anaerobically for growth of lactic acid bacteria and*Pediococcus*.

WL Differential and Nutrient Media contain a variety of substances to promote the growth and selection of bacteria and yeast. Yeast extract is a source of trace elements, vitamins and amino acids. Pancreatic digest of casein provides

nitrogen, amino acids, and carbon. Dextrose is the source of carbohydrate. Monopotassium phosphate buffers the media. Potassium chloride, calcium chloride, and ferric chloride are essential ions and help to maintain osmotic balance. Manganese sulfate and magnesium sulfate are sources of divalent cations. Bromcresol green is a pH indicator. Agar is the solidifying agent in WL Differential and WL Nutrient Media. Actidione (cycloheximide) is added as a selective agent in WL Differential Medium to inhibit the growth of yeasts and mold. Reliable counts for brewers' yeast are obtained with WL Nutrient Medium at pH 5.5. However, a pH adjustment to 6.5 is necessary for obtaining reliable counts of baker's and distiller's yeast.

FORMULA

WL Differential Medium	
Gram weight per liter:	80.0gm/L
Dextrose	50.0gm
Pancreatic Digest of Casein	5.0gm
Yeast Extract	4.0gm
Monopotassium Phosphate	0.55gm
Potassium Chloride	0.425gm
Calcium Chloride	0.125gm
Magnesium Sulfate	0.125gm
Bromcresol Green	0.022gm
Actidione	0.004gm
Ferric Chloride	0.0025gm
Manganese Sulfate	0.0025gm
Agar	20.0gm

Final pH 5.5 +/- 0.2 at 25ºC.

WL Nutrient Medium	
Gram weight per liter:	80.0gm/L
Dextrose	50.0gm
Pancreatic Digest of Casein	5.0gm
Yeast Extract	4.0gm
Monopotassium Phosphate	0.55gm
Potassium Chloride	0.425gm
Calcium Chloride	0.125gm
Magnesium Sulfate	0.125gm
Bromcresol Green	0.022gm
Ferric Chloride	0.0025gm

Manganese Sulfate	0.0025gm
Agar	20.0gm

Final pH 5.5 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original light beige with greenish tint.

Store the prepared culture media at 2-8ºC.

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

WL Differential Medium and WL Nutrient Medium

1. Suspend 82.1gm of the dehydrated culture media in 1 liter of distilled or deionized water.

2. Heat to boiling and mix to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 15 minutes.

Note: To adjust pH to 6.5, add the amount of 1% sodium carbonate solution specified on the product label to the rehydration water before dissolving the medium.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[1,3] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

1. Inoculate and incubate for 40-48 hours at 35ºC. for bacteria or 30ºC. for yeasts.

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.

WL Nutrient Medium can support the growth of bacteria, but unless the number of yeast cells on the plate is small the bacteria may not be detected.

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, and incubators, 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	
WL Differential Medium:					
Escherichia coli ATCC® 25922	A	40-48hr	30-35°C	Aerobic	Good growth
Lactobacillus fermentum ATCC® 9338	B	40-48hr	30-35°C	Anaerobic	Good growth
Saccharomyces cerevisiae ATCC® 9763	B	40-48hr	30-35°C	Aerobic	Inhibited
WL Nutrient Medium:					
Saccharomyces cerevisiae ATCC® 9763	B	40-48hr	30-35°C	Aerobic	Good growth
Lactobacillus fermentum ATCC® 9338	B	40-48hr	30-35°C	Aerobic	Fair to good growth
Escherichia coli ATCC® 25922	A	40-48hr	30-35°C	Aerobic	Fair to good 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™ WL Differential Medium and WL Nutrient Medium powder should appear homogeneous, free-flowing, and light beige, with a greenish tint, in color. The prepared media should appear slightly opalescent, without significant precipitate, and bluish-green in color.

REFERENCES

1. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

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

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

4. Green, S.R. and P.P. Gray. 1950. Paper read at American Society of Brewing Chemists Meeting. *Wallerstein Lab. Commun.*; 12:43.

5. Green, S.R. and P.P. Gray. 1950. A differential procedure applicable to bacteriological investigation in brewing. *Wallerstein Lab. Commun.*; 13:357.

6. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria,* Vol. I. Williams & Wilkins, Baltimore, MD.

7. Hall, J.F. 1971. *J. Inst. Brewing*; 77:513-516.

IFU-10293[B]

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

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

HDQA 2207B [D]