

Instructions for Use

BEER TESTING MEDIA

Cat. no. G93	Barney Miller Medium, 15x60mm Plate, 15ml	10 plates/bag
Cat. no. G182	Universal Beer Agar with Cycloheximide, 15x60mm Plate, 15ml	10 plates/bag
Cat. no. SRK95	EnviroTrans™ NaCl with Na Thiosulfate, 15x75mm Tube with swab, 5ml	20 vials/box
Cat. no. SRK100	EnviroTrans™ Barney Miller Broth, 15x75mm Tube, 5ml	20 vials/box

INTENDED USE

Hardy Diagnostics Beer Testing Media are used for the detection of beer spoilage microorganisms and for the cultivation and enumeration of bacteria and yeast encountered in the brewing industry.

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

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⁾ The medium contains a wide variety of nutrients, including tomato juice solids, yeast extract, peptonized milk, dextrose and salts. Beer is added to the heated agar base in order to approximate the environmental conditions present in the brewery. This composition ensures the 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 the growth of microbes specifically adapted to conditions in the brewery.^(4,7) Hardy Diagnostics Universal Beer Agar is supplemented with cycloheximide, which suppresses the growth of yeast and fungi, and further enhances the isolation of bacterial contaminants.

Barney Miller Medium was developed at the Miller Brewing Company by Barney, Kot, and Chicoye for the purpose of detecting and identifying beer spoilage microorganisms during beer manufacture.⁽³⁾ Lactic acid bacteria, such as *Lactobacillus* spp. and *Pediococcus* spp., often cause spoilage during brewing and processing.⁽⁵⁾ Though beer is not an ideal growth medium, lactic acid bacteria tend to flourish during beverage fermentation and maturation stages because they do not require oxygen for growth, are resistant to ethanol, and thrive at low pH. When present, lactic acid bacteria can cause excessive turbidity and acidity and disrupt the flavor development of the final product.⁽⁵⁾

Hardy Diagnostics Barney Miller Medium contains tomato juice broth, dipeptone and beef extract, which provide nitrogen, vitamins, carbon and minerals to optimize bacterial growth. Maltose and dextrose supply carbohydrates for fermentation. Potassium acetate and L-malic acid make the medium selective and L-cysteine HCl is a reducing agent and growth stimulant. Tween[®] 80 is used as a surfactant and agar is the solidifying agent.

EnviroTrans™ Barney Miller Broth is an enrichment medium for environmental monitoring of spoilage microorganisms in the brewing industry. It is used in combination with EnviroTrans™ NaCl with Na Thiosulfate (Cat. no. SRK95), which contains a swab used for surface sampling. After sampling, the swab is inserted into the

EnviroTrans™ Barney Miller Broth, which acts as an enrichment medium and has a similar composition to traditional Barney Miller Medium without the agar. Upon receipt in the lab, samples of the broth are plated to Barney Miller Medium (Cat. no. G93) or another suitable medium, where they can be analyzed for the presence and enumeration of beer spoilage microorganisms.

FORMULA

Ingredients per 750ml of deionized water:*

Universal Beer Agar with Cycloheximide:	
Dextrose	16.1gm
Peptonized Milk	15.0gm
Tomato Juice Solids	12.2gm
Yeast Extract	6.1gm
Dipotassium Phosphate	0.31gm
Monopotassium Phosphate	0.31gm
Magnesium Sulfate	0.12gm
Sodium Chloride	6.0mg
Ferrous Sulfate	6.0mg
Manganese Sulfate	6.0mg
Cycloheximide	0.01gm
Beer	250.0ml
Agar	12.0gm

Final pH 6.3 +/- 0.2 at 25°C.

Barney Miller Medium:	
Tomato Juice Broth	15.0gm
Maltose	15.0gm
Dextrose	10.0gm
Dipeptone	5.0gm
Potassium Acetate	3.0gm
Beef Extract	2.0gm
L-Malic Acid	0.5gm
Tween® 80	0.5gm
L-Cysteine HCl	0.2gm
Agar	15.0gm

EnviroTrans™ Barney Miller Broth (Cat. no. SRK100) is the same formula as Barney Miller Medium, but does not contain agar.

Final pH 5.6 +/- 0.1 at 25°C.

EnviroTrans™ NaCl with Na Thiosulfate:	
Sodium Chloride	8.5gm
Sodium Thiosulfate	0.4gm

Final pH 6.5 +/- 1.0 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light, except for Cat. no. SRK95 which should be stored at 2-30°C. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, 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

Allow the medium to come to room temperature prior to use. Consult listed references for more information on the correct procedure for use.⁽¹⁻¹⁰⁾

Universal Beer Agar with Cycloheximide may be used to detect and enumerate microbial spoilage organisms in pitching yeast, cooled wort or beer in storage.

Direct Plating Method of use:

1. Using an inoculum of the sample to be tested, perform a four quadrant streak to obtain well-isolated colonies. 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.

Barney Miller Medium may be used to detect and enumerate lactic acid beer spoilage microorganisms during beer manufacturing and processing.

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 the pour plate method.⁽¹⁾ Note: It is recommended that three replicates of each inoculum be performed, along with suitable blanks and controls of dilutions.
3. Incubate plates anaerobically at 28 degrees C. for up to 7 days and examine daily for growth of lactic acid bacteria.

EnviroTrans™ Barney Miller Broth is intended for use in environmental monitoring in the brewing industry.

Environmental Sampling Method of Use:

1. Aseptically collect surface samples using Hardy Diagnostics EnviroTrans™ NaCl with Na Thiosulfate (Cat. no. SRK95). Collect sample by rubbing the swab over the sample area (approximately 50cm²), reversing directions between strokes.
2. Repeat the collection procedure three more times, returning the swab head to the EnviroTrans™ NaCl with Na Thiosulfate after swabbing each area. When sampling utensils such as knives or ladles, run the swab over the entire surface of the instrument three times as described above.
3. Transfer swab to EnviroTrans™ Barney Miller Broth. If sample is not immediately taken to the lab, the sample can be refrigerated for up to 24 hours prior to analysis.
4. Prior to plating, shake each tube vigorously (50 cycles of 15cm in 10 seconds). Prepare plates using Barney Miller Medium or other appropriate medium, plating 1.0ml and 0.1ml samples of EnviroTrans™ Barney Miller Broth containing the sample. Incubate plates at 35 degrees C. for 40-48 hours, then calculate the number of colonies from 50cm² sample area.⁽¹¹⁾

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.

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.

Due to varying nutritional requirements, some strains may grow poorly or fail to grow at all on these media.

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, other culture media, swabs, applicator sticks, 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	
Universal Beer Agar with Cycloheximide (Cat. no. G182):					
<i>Lactobacillus acidophilus</i> ATCC® 314	A	40-48hr	35°C	Aerobic	Growth
<i>Lactobacillus fermentum</i> ATCC® 9338	A	40-48hr	35°C	Aerobic	Growth
<i>Acinetobacter baumannii</i> ATCC® 19606	A	40-48hr	35°C	Aerobic	Growth
Barney Miller Medium (Cat. no. G93):					
<i>Pediococcus damnosus</i> industrial strain	B	48-72hr	15-30°C	Anaerobic	Growth
<i>Saccharomyces cerevisiae</i> industrial strain	B	48hr	15-30°C	Aerobic	Growth
<i>Lactobacillus brevis</i> industrial strain	B	48hr	15-30°C	Aerobic	Growth
EnviroTrans™ Barney Miller Broth (Cat. no. SRK100):					
<i>Pediococcus damnosus</i> industrial strain	B	48hr	15-30°C	Anaerobic	Growth with yellow color change
<i>Saccharomyces cerevisiae</i> industrial strain	B	48hr	15-30°C	Aerobic	Growth with no color change
<i>Lactobacillus brevis</i> industrial strain	B	48hr	15-30°C	Aerobic	Growth with yellow color change

EnviroTrans™ NaCl with Na Thiosulfate is not a growth medium and is tested for sterility, pH, and fill only.

* 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.

PHYSICAL APPEARANCE

Universal Beer Agar and Universal Beer Agar with Cycloheximide should appear clear with a slight opalescence, and

medium to dark amber color.

Barney Miller Medium should appear clear with a slight opalescence, and light to medium amber color.

EnviroTrans™ Barney Miller Broth should appear trace to slightly hazy, and light to medium amber color.

EnviroTrans™ NaCl with Na Thiosulfate should appear clear and colorless.



Uninoculated plate of Universal Beer Agar with Cycloheximide (Cat. no. G182).



Lactobacillus acidophilus (ATCC® 314) growing on Universal Beer Agar with Cycloheximide (Cat. no. G182). Incubated aerobically for 24 hours at 35°C.

REFERENCES

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