

# Instructions for Use

## CRITERION™ CYTOPHAGA BROTH BASE

<a href="#">Cat. no. C7790</a>	CRITERION™ Cytophaga Broth Base	6gm
<a href="#">Cat. no. C7791</a>	CRITERION™ Cytophaga Broth Base	500gm
Cat. no. C7792	CRITERION™ Cytophaga Broth Base	2kg
Cat. no. C7793	CRITERION™ Cytophaga Broth Base	10kg
Cat. no. C7794	CRITERION™ Cytophaga Broth Base	50kg

### INTENDED USE

Hardy Diagnostics CRITERION™ Cytophaga Broth Base is recommended for the cultivation and maintenance of *Cytophaga* species and *Flavobacterium* species.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

### SUMMARY

Cold-water disease, bacterial gill disease (BGD), peduncle disease, columnaris, and other fish diseases commonly caused by bacterium in the *Cytophaga-Flavobacterium-Bacteroides* (CFB) group are usually associated with abrupt changes in environmental conditions such as extremes in water temperature, low dissolved oxygen concentration, overcrowding, poor diet, or improper handling. Many of these bacteria are ubiquitous in most, if not all, aquaculture environments and probably play a role in the turnover of organic matter, but some species may become opportunistic pathogens when fish are stressed.<sup>(1)</sup> Infection is most commonly associated with fry or fingerling populations and lesions may appear in various locations on different species of fish or may involve systemic infections with no visible signs of disease. Outbreaks can be highly contagious and, under the appropriate conditions, infection can spread rapidly, resulting in catastrophic losses and economic hardship in conservation hatcheries and the commercial aquaculture industry in a matter of days. Moreover, some bacteria can spread horizontally, from fish to fish, or vertically, from parent to offspring, and result in secondary infections or other diseases that can quickly overwhelm a population.

Researchers have devised many suitable low nutrient media in order to recover these etiological agents of disease, but Cytophaga Media has received the greatest acceptance.<sup>(2)</sup> Cytophaga Media is a general, non-selective medium that supports the growth of a wide variety of microorganisms; thus, knowledge of cell and colony morphology is essential.<sup>(3)</sup> Species in the *Cytophaga-Flavobacterium-Bacteroides* (CFB) group generally exhibit gram-negative, rod shaped cells of varying length and width, produce a yellow, orange, or brick red pigment and may swarm by gliding or produce a biofilm. The low nutrient concentration of this medium mimics the natural habitat conditions of these opportunistic pathogens and helps promote their isolation.

Hardy Diagnostics CRITERION™ Cytophaga Broth Base contains casein peptone, yeast extract and beef extract which provide essential amino acids and other nitrogenous compounds. Sodium acetate is the sodium salt of acetic acid and is

used as a buffering agent. The media can be enriched with ingredients (e.g. serum, gelatin and starch) for fastidious microorganisms or supplemented with selective agents to make Selective Cytophaga Agar (SCA) for greater isolation of *Cytophaga* species and *Flavobacterium columnare* from mixed cultures. It can also be prepared with 70% seawater for the cultivation and maintenance of marine microorganisms like *Flexibacter maritimus*, or other marine bacteria recognized as global contributors to diseases in salmon.<sup>(1,7)</sup>

## FORMULA\*

Gram weight per liter:	3.0gm/L
Casein Peptone	1.0gm
Yeast Extract	1.0gm
Beef Extract	0.6gm
Sodium Acetate	0.4gm

Final pH 7.0 +/- 0.2 at 25°C.

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

## STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30°C. 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 beige

Store the prepared culture media at 2-8°C.

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 3.0gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.

Note: At least 70% sterile seawater should be used for the optimum cultivation of marine microorganisms. The addition of inorganic salts such as KCl and NaCl may be needed to promote the growth of marine species.

2. Check pH and adjust if necessary.

3. Dispense into appropriate containers.

4. Sterilize in the autoclave at 121°C. for 15 minutes.

## PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references.

## 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.

Organisms belonging to the *Cytophaga-Flavobacterium-Bacteroides* (CFB) group are organotrophs and may be either aerobic, microaerophilic, capnophilic (CO<sub>2</sub> requiring), or facultatively anaerobic.<sup>(1)</sup> Appropriate incubation procedures should be followed for best results.

*F. branchiophilum* are fastidious and enrichment of the medium with serum, gelatin or starch is recommended.<sup>(13)</sup>

Columnaris bacteria are best grown on selective media such as Selective Cytophaga Agar containing neomycin (5mg/L) and polymyxin B (200 units/mL), whereas most other fish pathogens and aquatic bacteria are inhibited.<sup>(4)</sup>

*Flexibacter maritimus* will only grow on media prepared with at least 50% seawater. In addition, the bacterium requires the use of two inorganic salts (KCl and NaCl) for optimum growth.

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, antibiotics, sea water, inorganic salts, serum, gelatin, starch and incubators, etc., are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests every lot for pH and quality specifications as outlined on the Certificates of Analysis (CofA).

## 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™ Cytophaga Broth Base powder should appear homogeneous, free-flowing, and beige in color. The prepared media should appear clear, and light beige in color.

## REFERENCES

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1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: [HardyDiagnostics.com](http://HardyDiagnostics.com)

Email: [TechnicalServices@HardyDiagnostics.com](mailto:TechnicalServices@HardyDiagnostics.com)

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