



## Instructions for Use

### BETA TOXIN

<a href="#">Cat. no. Z306</a>	Beta Toxin	25ml
<a href="#">Cat. no. Z305</a>	Beta Toxin	100ml

### INTENDED USE

Hardy Diagnostics Beta Toxin is used to detect the presence of the CAMP factor gene which aids in the presumptive identification of *Streptococcus agalactiae* from bovine samples.

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

### SUMMARY

Bovine mastitis, the inflammation of the mammary gland in dairy cattle, is mainly caused by infections from bacteria. Of the 130 microorganisms isolated from bovine mastitic milk, *Streptococcus* species are the most frequent mastitis pathogens.<sup>(6)</sup> Often these organisms are associated with chronic subclinical mastitis with occasional episodes of acute or subacute clinical mastitis. Streptococcal isolates most commonly encountered in mastitis diagnostic laboratories are *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus bovis*, and Lancefield group G. Of these organisms, *Streptococcus agalactiae* is the most common cause of subclinical bovine mastitis infections and is the etiological agent of more than 40% of all mastitis infections.<sup>(5)</sup> Beta Toxin is used in the presumptive identification of *Streptococcus agalactiae* from bovine samples based on a positive CAMP reaction.

Traditionally, the CAMP test is performed by streaking a Blood Agar plate with a beta-hemolysin-producing strain of *Staphylococcus aureus*. *Streptococcus agalactiae* is then streaked on the plate perpendicular to the *S. aureus* streak. *Streptococcus agalactiae* secretes CAMP factor, an extracellular protein, that interacts with the beta-hemolysin secreted by *S. aureus*. This interaction produces a synergistic effect and as a result enhanced hemolysis is observed at the juncture of the two organisms. Enhanced hemolysis is indicated by an arrowhead shaped zone of beta-hemolysis.<sup>(3)</sup> However, streaking *S. aureus* cultures on diagnostic plates can be problematic. Isolation of suspected colonies and restreaking on a conventional CAMP plate is time consuming and expensive.<sup>(8)</sup>

Hardy Diagnostics Beta Toxin is a simplified and modified version of the traditional CAMP procedure. Beta Toxin contains extracted *S. aureus* beta-hemolysin and can be used to evaluate the CAMP reaction on primary isolation. The modified CAMP procedure has demonstrated a high degree of reliability in the identification of *S. agalactiae*.<sup>(8)</sup> In this convenient method, Beta Toxin is swabbed in a single line across Bovine Blood Agar (Cat. no. A188). Once the Beta Toxin has been absorbed into the media, the plate is inoculated with the milk sample. After overnight incubation, the plate is observed for enhanced hemolysis indicative of *S. agalactiae*.<sup>(8)</sup> Alternatively, the use of Bovine Blood Agar with Esculin (Cat. no. A189) can be used to presumptive identification of *S. agalactiae* from rare CAMP-positive isolates of *S. uberis*. This media contains esculin, which distinguishes positive esculin-hydrolysis species (*S. uberis*) from negative esculin-hydrolysis species (*S. agalactiae*). When esculin is hydrolyzed, it reacts with a compound in the media to produce a darkening or blackness around the colonies.<sup>(6-8)</sup>

## FORMULA

Beta Toxin contains filtered beta-hemolysin prepared from beta-hemolytic staphylococci.

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at -20 to +8°C away from direct light. It is recommended that unopened bottles be stored frozen for long-term storage. Product should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light and excessive heat.

The expiration dating 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 quality control incubation times.

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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at [www.cdc.gov/ncidod/dhqp/gl\\_isolation.html](http://www.cdc.gov/ncidod/dhqp/gl_isolation.html).

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

Refer to the document [SDS Search](#) instructions on the Hardy Diagnostics' website for more information.

## PROCEDURE

Sample Collection: It is important that a milk sample be taken to ensure that the potential pathogens origin was from the mammary gland and not from dust or fecal particles on the udder surface. To ensure the contaminant is from the milk, the teat surface and orifice should be wiped with seventy percent (70%) ethyl alcohol. It is also essential to obtain a sample before the cow has been treated with antimicrobial agents. Consult listed references for additional information on specimen collection.<sup>(6)</sup>

1. Warm Beta Toxin to room temperature.
2. Dip a sterile cotton swab into the Beta Toxin. Ensure that the swab is saturated with Beta Toxin.
3. Inoculate the dried surface of Bovine Blood Media (Cat. no. A188 or A189) with the saturated swab by streaking the swab in the desired pattern. See listed references for accepted inoculation procedures for Beta Toxin application.<sup>(5-8)</sup> If the surface of the media shows excess moisture (droplets on the surface of the media or on the petri plate lid), then incubate the plates for 10 to 30 minutes with the lids ajar prior to swabbing the media surface.

4. Repeat this swab streaking procedure with a second swab to ensure an even distribution of Beta Toxin.
5. Allow media swabbed with Beta Toxin to dry prior to inoculating with the sample to be tested. Drying of Beta Toxin prepared plates can be accelerated by incubating the swabbed media prior to use.
6. Media prepared with Beta Toxin should be inoculated with the milk sample according to accepted procedures described in the listed reference texts.<sup>(5-8)</sup>
7. After 18-24 hours of incubation, observe plates for growth and enhanced beta-hemolysis.

## INTERPRETATION OF RESULTS

On Bovine Blood Agar (Cat. no. A188), a positive CAMP reaction is indicated by observing enhanced beta-hemolysis in the presence of Beta Toxin. A positive CAMP reaction is indicative of *S. agalactiae*. A negative CAMP test is indicated when enhanced hemolysis is not observed in the presence of Beta Toxin.

On Bovine Blood Agar with Esculin (Cat. no. A189) rare CAMP-positive isolates of *S. uberis* can be differentiated from CAMP-positive strains of *S. agalactiae*. CAMP-positive isolates which are surrounded by a darkening or blackening of the media, indicative of esculin hydrolysis, can presumptively be identified as *S. uberis*. CAMP-positive isolates which are not surrounded by a darkening or blackening of the media can be presumptively identified as *S. agalactiae*.

## LIMITATIONS

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

This product is used in conjunction with other tests to identify cultures of *Streptococcus agalactiae*. It is necessary to confirm, with other biochemical tests, the identification of all organisms suspected of being *Streptococcus agalactiae*.

Rare strains of *Streptococcus uberis* may produce a weakly positive CAMP reaction. However, the use of Bovine Blood Agar with Esculin (Cat. no. A189) can be used to aid in the differentiation of *S. agalactiae* from rare CAMP-positive isolates of *S. uberis*. This media contains esculin and distinguishes positive esculin-hydrolysis species (*S. uberis*) from negative esculin-hydrolysis species (*S. agalactiae*).<sup>(6-8)</sup>

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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Streptococcus agalactiae</i> ATCC <sup>®</sup>	*	18-24hr	35°C	Aerobic	Growth; enhanced beta-hemolysis in the presence of Beta Toxin

13813					
<i>Streptococcus dysgalactiae</i> ATCC® 43078	*	18-24hr	35°C	Aerobic	Growth; no enhanced beta-hemolysis in the presence of Beta Toxin

\*See Procedure section.

## 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 certificates of analysis (CofA) available from Hardy Diagnostics [Certificates of Analysis](#) website. In addition, refer to the following document "[Finished Product Quality Control Procedures](#)," for more information on QC or see reference(s) for more specific information.

## PHYSICAL APPEARANCE

Beta Toxin should appear clear, and light amber in color.



*Streptococcus agalactiae* (ATCC® 13813) colonies growing on Bovine Blood Agar (Cat. No. A188) showing enhanced beta-hemolysis in the presence of Beta Toxin (Cat. no. Z306). Incubated aerobically for 24 hours at 35°C.



*Streptococcus dysgalactiae* (ATCC® 43078) colonies growing on Bovine Blood Agar (Cat. No. A188) in the presence of Beta Toxin (Cat. no. Z306) without enhanced beta-hemolysis. Incubated aerobically for 24 hours at 35°C.

## REFERENCES

1. Versalovic, J., et al. *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, D.C.
2. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
3. American Public Health Association. *Standard Methods for the Examination of Dairy Products*, APHA, Washington, D.C.
4. Quinn, P.J., et al. 1994. *Clinical Veterinary Microbiology*. Wolfe Publishing, London, England.
5. National Mastitis Council. 1999. *Laboratory Handbook on Bovine Mastitis*. NMC, Inc., Madison, WI.

6. Carter, G.R., et al. 1995. *Essentials of Veterinary Microbiology* , 5th ed. Williams & Wilkins, Philadelphia, PA.

7. Jasper, D.E., et al. 1968. Use of Crude beta-Staphylococcal Hemolysin for the Presumptive Recognition of *Streptococcus agalactiae*. *Am. J. of Vet. Clin. Path.*; 2:43-47.

ATCC is a registered trademark of the American Type Culture Collection.

IFU-10065[A]



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