

Bacto™ Casitone • Trypticase™ Peptone

Bacto™ Tryptone • BiTek™ Tryptone

Intended Use

Ingredients, where noted, conform with specifications of *The United States Pharmacopeia (USP)*.

Bacto Casitone, Trypticase Peptone, Bacto Tryptone and BiTek Tryptone are used in preparing microbiological culture media.

Summary and Explanation

The manufacturing process for an enzymatic digest of casein is not as destructive as an acid hydrolysis. Thus, the casein is not broken down as completely into its constituent components. In many cases this makes for a more nutritious hydrolysate, especially for those organisms that prefer peptides to amino acids.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Bacto™ Casitone

Dehydrated Appearance: Tan, free-flowing, granules.

Solution: 1.0%, 2.0% and 10.0 % solutions, soluble in purified water. 1.0% solution is light amber, clear. 2.0% solution is light to medium amber, clear, may have a slight precipitate. 10.0% solution is medium to dark amber, clear to very slightly opalescent, may have a precipitate.

Reaction of 1.0%
Solution at 25°C: pH 6.8-7.4

Bacto™ Tryptone

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is very light to light amber, clear. 2.0% solution is light to medium amber, clear. 10.0% solution is medium to dark amber, clear to slightly opalescent, may have a slight precipitate.

Reaction of 2.0%
Solution at 25°C: pH 6.5-7.5

BiTek™ Tryptone

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 1.0%, 2.0% and 10.0% solutions, soluble in purified water. 1.0% solution is very light to light amber, clear. 2.0% solution is light to medium amber, clear. 10.0% solution is medium to dark amber, clear to slightly opalescent, may have a slight precipitate.

Reaction of 2.0%
Solution at 25°C: pH 7.2 ± 0.2

Cultural Response

Biochemical Reactions

Bacto™ Casitone, Bacto™ Tryptone or BiTek™ Tryptone

Prepare a sterile solution as directed below. Adjust final pH to 7.2-7.4. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT
Fermentable Carbohydrates	2%	<i>Escherichia coli</i>	25922	~10 ⁷	Negative
Indole Production	0.1%	<i>Escherichia coli</i>	29552	0.1 mL, undiluted	Positive
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	<i>Enterobacter aerogenes</i>	13048	0.1 mL, undiluted	Positive
Hydrogen Sulfide Production	1%	<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	0.1 mL, undiluted	Positive

Growth Response

Bacto™ Casitone, Bacto™ Tryptone or BiTek™ Tryptone

Prepare a sterile solution with 2.0% Bacto Casitone, Bacto Tryptone or BiTek Tryptone, 0.5% sodium chloride and 1.5% agar. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Brucella suis</i>	4314*	Undiluted	Good
<i>Escherichia coli</i>	25922	30-300	Good
<i>Staphylococcus aureus</i>	25923	30-300	Good

*If this strain is not available, verify performance with a known strain.

Continued

Identity Specifications**BBL™ Trypticase™ Peptone**

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.
 Solution: 2.0% solution, soluble in purified water. Solution is clear to slightly hazy.
 Reaction of 2.0% Solution at 25°C: pH 6.5-7.5

Cultural Response**Biochemical Reactions****BBL™ Trypticase™ Peptone**

Prepare a sterile solution as directed below. Adjust final pH to 7.2-7.4. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

TEST	TEST SOLUTION	ORGANISM	ATCC™	INOCULUM CFU	RESULT
Fermentable Carbohydrates	2%	<i>Escherichia coli</i>	29552	~10 ⁷	Negative
Indole Production	0.1%	<i>Escherichia coli</i>	29552	0.1 mL, undiluted	Positive
Acetylmethylcarbinol Production	0.1% with 0.5% dextrose	<i>Enterobacter aerogenes</i>	13048	0.1 mL, undiluted	Positive
Hydrogen Sulfide Production	1%	<i>Citrobacter freundii</i>	8454	0.1 mL, undiluted	Positive

Growth Response**BBL™ Trypticase™ Peptone**

1. Prepare a sterile solution of peptone agar without (plain) and with 5% sheep blood (SB) using 10 g **Trypticase** Peptone, 2.5 g sodium chloride and 6.5 g agar in 500 mL of purified water. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 3 days (incubate streptococci with CO₂).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB
<i>Enterobacter aerogenes</i>	13048	10 ³ -10 ⁴	Good	N/A
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	Good	N/A
<i>Staphylococcus aureus</i>	6538P	10 ³ -10 ⁴	Good	N/A
<i>Staphylococcus epidermidis</i>	12228	10 ³ -10 ⁴	Good	N/A
<i>Streptococcus agalactiae</i>	12386	10 ³ -10 ⁴	N/A	Good, beta hemolysis
<i>Streptococcus pneumoniae</i>	6305	10 ³ -10 ⁴	N/A	Good, alpha hemolysis
<i>Streptococcus pyogenes</i>	49117	10 ⁴ -10 ⁵	Good	Good, beta hemolysis

2. Prepare a sterile solution of chocolate peptone agar using **Trypticase** Peptone. Adjust final pH to 7.2-7.4. Inoculate and incubate plates at 35 ± 2°C for 3 days with CO₂.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Neisseria gonorrhoeae</i>	19424	10 ³ -10 ⁴	Good

Bacto Casitone can be used as a component in microbiological culture media or in fermentation applications. A recent publication has also reported that the stability of lyophilized influenza virus vaccine was augmented by the addition of 2% Casitone.¹

Trypticase Peptone is the primary nitrogen source in **Trypticase** Soy Broth and Agar. This product is recommended for use in media formulations, where good growth of fungi and bacteria is required. **Trypticase** Peptone is referenced in *Official Methods of Analysis of AOAC International* and meets specifications in the *USP* for pancreatic digest of casein.^{2,3}

Bacto Tryptone was developed by Difco Laboratories while investigating a peptone particularly suitable for the elaboration of indole by bacteria. It is also notable for the absence of detectable levels of carbohydrates. **Bacto** Tryptone has been used in conjunction with casamino acids in nutritional studies to determine amino acids vs. peptide utilization.^{4,5} It is included in standard methods applications and is listed in the reagent section of the *USP* as meeting the specifications for pancreatic digest of casein, a component in many of the media listed.^{2,3,6-11} The *European Pharmacopoeia* also lists pancreatic digest of casein as a component in many of the recommended media.¹² **Bacto** Tryptone also works well in fermentation applica-

tions. It has been used successfully with commonly used organisms, such as *Escherichia coli*,¹³ as well as uncommon organisms, such as the diatom *Nitzschia laevis*.¹⁴

BiTek Tryptone is prepared similarly to **Bacto** Tryptone but the final product goes through fewer refinement steps during processing. This product provides some of the same benefits as **Bacto** Tryptone in instances where a less refined hydrolysate can be utilized.

Principles of the Procedure

Bacto Casitone, **Trypticase** Peptone, **Bacto** Tryptone and **BiTek** Tryptone are pancreatic digests of casein. Casein is the main milk protein and a rich source of amino acid nitrogen.

Typical Analysis

Refer to Product Tables in the Reference Guide section of this manual.

Precautions¹⁵

1. Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with clinical specimens of human or animal origin containing or potentially containing pathogenic *Brucella* spp.

2. Biosafety Level 3 practices, containment equipment and facilities are recommended for all manipulations of cultures of the pathogenic *Brucella* spp. and for experimental animal studies.

Directions for Preparation from Dehydrated Product

Refer to the final concentration of Bacto Casitone, Trypticase Peptone, Bacto Tryptone and BiTek Tryptone in the formula of the medium being prepared. Add appropriate product as required.

Procedure

See appropriate references for specific procedures using Bacto Casitone, Trypticase Peptone, Bacto Tryptone and BiTek Tryptone.

Expected Results

Refer to appropriate references and procedures for results.

References

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2. Horowitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, Md.
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5. Nagel, Oostra, Tramper and Rinzema. 1999. Process Biochem. 35: 69.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
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9. Marshall (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington D.C.
10. U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. EPA-821/R-97/004. Office of Water, Washington, D.C.
11. U.S. Department of Agriculture. 1998. Microbiology laboratory guidebook, 3rd ed. Food Safety and Inspection Service, USDA, Washington, D.C.
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13. Sivakesavs, Chen, Hackett, Huang, Lam, Lam, Siu, Wong and Wong. 1999. Process Biochem. 34:893.
14. Wen and Chen. 2001. Enzyme Microbia Technol. 29:341.
15. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 1999. Biosafety in microbiological and biomedical laboratories, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

Bacto™ Casitone

COMPF SMD SMWW USDA

Cat. No. 225930 Dehydrated – 500 g
225910 Dehydrated – 10 kg

BBL™ Trypticase™ Peptone

AOAC BAM COMPF EP EPA SMD SMWW USDA USP

Cat. No. 211921 Dehydrated – 454 g
211922 Dehydrated – 5 lb (2.3 kg)
211923 Dehydrated – 25 lb (11.3 kg)

Bacto™ Tryptone

AOAC BAM COMPF EP EPA SMD SMWW USDA USP

Cat. No. 211705 Dehydrated – 500 g
211699 Dehydrated – 2 kg

BiTek™ Tryptone

Cat. No. 251420 Dehydrated – 10 kg

Casman Agar Base

Intended Use

Casman Agar Base is used for the cultivation of fastidious pathogenic organisms, such as *Haemophilus influenzae* and *Neisseria gonorrhoeae*, from clinical specimens.

Summary and Explanation

Members of the genus *Haemophilus* are fastidious microorganisms that require the addition of X and/or V growth factors for *in vitro* cultivation.¹ *Neisseria* are also fastidious microorganisms with complex growth requirements.²

In 1947, Casman described a blood-enriched medium prepared without an infusion of fresh meat for cultivation of *Haemophilus* and gonococci.¹ The medium was developed to replace previous formulations that required time-consuming preparations of fresh and heated blood and fresh meat infusion to supply the nutrients necessary for the growth of these fastidious organisms.^{2,3}

Casman found that nicotinamide interfered with the activity of an enzyme in blood that inactivates V factor (NAD). Using unheated human blood, he found that amount of nicotinamide required for good growth of *H. influenzae* was inhibitory to gonococci.² Therefore, he reduced the nicotinamide to a level that allowed good growth of gonococci.

User Quality Control

Identity Specifications

BBL™ Casman Agar Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.3% solution, soluble in purified water upon boiling. Solution is medium to dark, yellow to tan, hazy to cloudy, with a moderate to large amount of cream flocculation.
Prepared Appearance:	Medium to dark, yellow to tan, hazy to cloudy, with a moderate to large amount of cream flocculation.
Reaction of 4.3% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Casman Agar Base

Prepare the medium per label directions. Inoculate and incubate for 42-48 hours at 35 ± 2°C, aerobically for *L. monocytogenes* and with 3-5% CO₂ for all other organisms.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS
<i>Haemophilus influenzae</i>	10211	10 ² -10 ³	Good	N/A
<i>Haemophilus parahaemolyticus</i>	10014	10 ² -10 ³	Good	Beta
<i>Listeria monocytogenes</i>	19115	10 ² -10 ³	Good	Weak beta
<i>Neisseria gonorrhoeae</i>	43070	10 ² -10 ³	Good	N/A
<i>Streptococcus pyogenes</i>	19615	10 ² -10 ³	Good	Beta