
Bacto™ Tryptic Soy Broth/Trypticase™ Soy Broth (Soybean-Casein Digest Medium) Trypticase™ Soy Broth with 6.5% Sodium Chloride Trypticase™ Soy Broth with 5% Fildes Enrichment Bacto™ Tryptic Soy Broth without Dextrose

Intended Use

Tryptic (Trypticase) Soy Broth (Soybean-Casein Digest Medium) is a general purpose medium used in qualitative procedures for the cultivation of fastidious and nonfastidious microorganisms from a variety of clinical and nonclinical specimens.

Trypticase Soy Broth with 6.5% Sodium Chloride is used to differentiate *Enterococcus* spp. from the *Streptococcus bovis* group of streptococci.

Trypticase Soy Broth with 5% Fildes Enrichment is used for the cultivation of fastidious organisms; e.g., *Haemophilus influenzae*.

Tryptic Soy Broth without Dextrose, a low carbohydrate formulation of Tryptic Soy Broth, is used for cultivating fastidious and nonfastidious microorganisms.

Tryptic (Trypticase) Soy Broth meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*¹⁻³ performance specifications, where applicable.

Summary and Explanation

Tryptic (Trypticase) Soy Broth (TSB) is a nutritious medium that will support the growth of a wide variety of microorganisms, including common aerobic, facultative and anaerobic bacteria and fungi.⁴⁻⁷ This formulation is included in the *USP* as a medium for use in performing microbial enumeration tests and tests for specified microorganisms when testing nonsterile pharmaceutical products.¹

TSB was chosen by the USDA Animal and Plant Health Inspection Service for detecting viable bacteria in live vaccines.⁸ TSB is recommended for testing bacterial contaminants in cosmetics^{9,10} and complies with established standards in the food industry.¹⁰⁻¹⁶

Because of its capacity for growth promotion, TSB is also recommended for use as the inoculum broth for disc diffusion and agar dilution antimicrobial susceptibility testing as standardized by the Clinical and Laboratory Standards Institute (CLSI).^{17,18}

Trypticase Soy Broth with 6.5% Sodium Chloride is used to differentiate the enterococcal species from the *S. bovis* group of streptococci by the 6.5% NaCl tolerance test.¹⁹

Trypticase Soy Broth supplemented with 5% Fildes Enrichment provides growth factors necessary for the cultivation of fastidious organisms.²⁰

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™/Bacto™** 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™ Tryptic Soy Broth

Dehydrated Appearance: Light beige, free-flowing, homogeneous.
Solution: 3.0% solution, soluble in purified water upon warming. Solution is light amber, clear.
Prepared Appearance: Light amber, clear.
Reaction of 3.0% Solution at 25°C: pH 7.3 ± 0.2

Difco™ Tryptic Soy Broth (prepared bottles)

Appearance: Light to medium tan yellow, clear to trace hazy.
Reaction at 25°C: pH 7.3 ± 0.2

Bacto™ Tryptic Soy Broth without Dextrose

Dehydrated Appearance: Light beige, free-flowing, homogeneous.
Solution: 2.75% solution, soluble in purified water upon warming. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance: Light amber, clear to very slightly opalescent.
Reaction of 2.75% Solution at 25°C: pH 7.3 ± 0.2

Cultural Response

Bacto™ Tryptic Soy Broth

Prepare the medium per label directions. Inoculate and incubate at 30-35°C for 18-72 hours (up to 5 days for *A. brasiliensis* and *C. albicans*). Prepare duplicate cultures of *A. brasiliensis*, *B. subtilis* and *C. albicans* and incubate at 20-25°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Neisseria meningitidis</i>	13090	10-100	Fair to good
<i>Staphylococcus epidermidis</i>	12228	10-100	Good
<i>Streptococcus pneumoniae</i>	6305	10-100	Good
<i>Streptococcus pyogenes</i>	19615	10-100	Good
<i>Aspergillus brasiliensis</i> (niger)	16404	<100	Growth (30-35°C)
<i>Aspergillus brasiliensis</i> (niger)	16404	<100	Growth (20-25°C)
<i>Bacillus subtilis</i>	6633	<100	Growth (30-35°C)
<i>Bacillus subtilis</i>	6633	<100	Growth (20-25°C)
<i>Candida albicans</i>	10231	<100	Growth (30-35°C)
<i>Candida albicans</i>	10231	<100	Growth (20-25°C)
<i>Escherichia coli</i>	8739	<100	Growth
<i>Pseudomonas aeruginosa</i>	9027	<100	Growth
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	<100	Growth
<i>Staphylococcus aureus</i>	6538	<100	Growth



Difco™ Tryptic Soy Broth (prepared bottles)

Inoculate and incubate at 30-35°C for 18-24 hours (up to 3 days for *B. subtilis*). For (*) cultures incubate at 20-25°C for up to 3 days (up to 5 days for *A. brasiliensis*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Aspergillus brasiliensis</i> (niger)*	16404	10-100	Growth (20-25°C)
<i>Bacillus subtilis</i>	6633	10-100	Growth (30-35°C)
<i>Bacillus subtilis</i> *	6633	10-100	Growth (20-25°C)
<i>Candida albicans</i> *	10231	10-100	Growth (20-25°C)
<i>Escherichia coli</i>	8739	10-100	Growth
<i>Pseudomonas aeruginosa</i>	9027	10-100	Growth
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10-100	Growth
<i>Staphylococcus aureus</i>	6538	10-100	Growth

Bacto™ Tryptic Soy Broth without Dextrose

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Neisseria meningitidis</i>	13090	30-300	Fair to good
<i>Staphylococcus epidermidis</i>	12228	30-300	Good
<i>Streptococcus pneumoniae</i>	6305	30-300	Good
<i>Streptococcus pyogenes</i>	19615	30-300	Good

Continued

Tryptic Soy Broth without Dextrose, a modification of TSB, is a basal medium to which carbohydrates may be added for use in fermentation studies. Phenol red and other indicators may also be added.

Principles of the Procedure

Enzymatic digests of casein and soybean provide amino acids and other complex nitrogenous substances. Dextrose is an energy source. Sodium chloride maintains the osmotic equilibrium. Dibasic potassium phosphate acts as a buffer to control pH.

Identity Specifications

BBL™ Trypticase™ Soy Broth

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 3.0% solution, soluble in purified water upon warming. Solution is light, tan to yellow, clear to slightly hazy.

Prepared Appearance: Light, tan to yellow, clear to slightly hazy.

Reaction of 3.0%

Solution at 25°C: pH 7.3 ± 0.2

BBL™ Trypticase™ Soy Broth (prepared bottles)

Appearance: Light to medium tan yellow, clear to trace hazy.

Reaction at 25°C: pH 7.3 ± 0.2

Cultural Response

BBL™ Trypticase™ Soy Broth

Prepare the medium per label directions. Inoculate tubes and incubate at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*). Prepare duplicate cultures of *A. brasiliensis*, *B. subtilis* and *C. albicans* and incubate at 20-25°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Aspergillus brasiliensis</i> (niger)	16404	<100	Growth (30-35°C)
<i>Aspergillus brasiliensis</i> (niger)	16404	<100	Growth (20-25°C)
<i>Bacillus subtilis</i>	6633	<100	Growth (30-35°C)
<i>Bacillus subtilis</i>	6633	<100	Growth (20-25°C)
<i>Candida albicans</i>	10231	<100	Growth (30-35°C)
<i>Candida albicans</i>	10231	<100	Growth (20-25°C)
<i>Escherichia coli</i>	8739	<100	Growth
<i>Pseudomonas aeruginosa</i>	9027	<100	Growth
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	<100	Growth
<i>Staphylococcus aureus</i>	6538	<100	Growth

BBL™ Trypticase™ Soy Broth (prepared bottles)

Inoculate and incubate at 35-37°C for 48 hours. Incubate (*) cultures at 30-35°C for up to 3 days. Incubate (**) cultures at 20-25°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Escherichia coli</i>	25922	<100	Growth
<i>Staphylococcus aureus</i>	25923	<100	Growth
<i>Aspergillus brasiliensis</i> (niger)**	16404	<100	Growth (20-25°C)
<i>Bacillus subtilis</i> *	6633	<100	Growth (30-35°C)
<i>Bacillus subtilis</i> **	6633	<100	Growth (20-25°C)
<i>Candida albicans</i> **	10231	<100	Growth (20-25°C)
<i>Pseudomonas aeruginosa</i> *	9027	<100	Growth (30-35°C)
<i>Staphylococcus aureus</i> *	6538	<100	Growth (30-35°C)

The addition of 6.5% sodium chloride to Trypticase Soy Broth permits the differentiation of salt-tolerant enterococci, which are resistant to the high salt content, from the salt-intolerant *S. bovis* group and other streptococcal species. At this concentration, the sodium chloride is a selective agent that interferes with membrane permeability and osmotic and electrokinetic equilibria.⁴

Fildes Enrichment is a peptic digest of sheep blood that supplies the X (hemin) and V (nicotinamide adenine dinucleotide, NAD) factors necessary for the growth of *H. influenzae*.

Dextrose is omitted from the formula for Tryptic Soy Broth without Dextrose to permit use of the medium in fermentation studies. The carbohydrate concentration used most frequently in fermentation reactions is 0.5% or 1%.

Tryptic Soy Broth and Trypticase Soy Broth are provided as prepared media in a variety of bottle styles. In addition, Tryptic Soy Broth is provided as a Sterile Pack Bottle; i.e., the bottle has been terminally sterilized inside of autoclavable double-bags. All varieties of bottled TSB conform with requirements for Ready-To-Use Media as described in the USP.

Formulae

Bacto™ Tryptic Soy Broth (Soybean-Casein Digest Medium)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Papaic Digest of Soybean	3.0	g
Dextrose	2.5	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g

BBL™ Trypticase™ Soy Broth (Soybean-Casein Digest Broth)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Papaic Digest of Soybean	3.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g
Dextrose	2.5	g

Bacto™ Tryptic Soy Broth without Dextrose

Approximate Formula* Per Liter

Pancreatic Digest of Casein	17.0	g
Enzymatic Digest of Soybean Meal	3.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	2.5	g

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
Bacto™ Tryptic Soy Broth – 30 g;
BBL™ Trypticase™ Soy Broth – 30 g;
Bacto™ Tryptic Soy Broth without Dextrose – 27.5 g.
 Mix thoroughly.
2. Warm gently until solution is complete.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.^{5,7,17-19}

For food, dairy or cosmetic samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.⁹⁻¹⁶

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.¹

Procedure

For clinical specimens, refer to appropriate standard references for details on testing protocol to obtain isolated colonies from specimens using Tryptic/Trypticase Soy Broth.¹⁷⁻¹⁹

For food, dairy or cosmetic samples, refer to appropriate standard references for details on test methods using Tryptic/Trypticase Soy Broth.⁹⁻¹⁶

For pharmaceutical samples, refer to *USP* General Chapters <61> and <62> for details on the examination of nonsterile products and performing microbial enumeration tests and tests for specific organisms using Tryptic/Trypticase Soy Broth.¹

Swab specimens may be inserted into the medium after inoculation of appropriate plated media. For liquid specimens, use a sterile inoculating loop to transfer a loopful of the specimen to the broth medium. Specimens known or suspected to contain obligate anaerobes should be inoculated near the bottom of the tube.

Incubate the tubes and bottles with loosened caps at 35 ± 2°C aerobically with or without supplementation with carbon dioxide. Tubed and bottled media intended for the cultivation of anaerobes should be incubated under anaerobic conditions. An efficient and easy way to obtain suitable anaerobic conditions is through the use of **BD GasPak™ EZ** anaerobic systems or equivalent alternative system. Examine for growth after 18-24 hours and 42-48 hours of incubation.

Expected Results

Growth in broth media is indicated by the presence of turbidity compared to an uninoculated control. Broth cultures should be held for at least a week before discarding as negative.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
5. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby Inc., St. Louis, Mo.
6. Fredette and Forget. 1961. The sensitivity of several media to small inocula. Extract from a paper presented at the Canadian Society of Microbiology Annual Meeting, June 12-15. Kingston, Ontario, Canada.
7. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
8. Federal Register. 1992. Fed. Regist. 21:113.26.
9. Curry, Joyce and McEwen. 1993. CTFA microbiology guidelines. The Cosmetic, Toiletry and Fragrance Association, Inc., Washington, D.C.
10. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
11. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
12. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
13. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
14. Health Canada. The compendium of analytical methods, online. Food Directorate, Health Products and food Branch, Health Canada, Ottawa, Ontario Canada.
15. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.
16. International Organization for Standardization. 1996. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method. ISO 11290-1, 1st ed., 1996-12-15. ISO, Geneva, Switzerland.
17. Clinical and Laboratory Standards Institute. 2006. Approved Standard M2-A9: Performance standards for antimicrobial disk susceptibility tests, 9th ed., CLSI, Wayne, Pa.
18. Clinical and Laboratory Standards Institute. 2006. Approved Standard M7-A7: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed., CLSI, Wayne, Pa.
19. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
20. Fildes. 1920. Br. J. Exp. Pathol. 1:129.

Availability

Bacto™ Tryptic Soy Broth (Soybean-Casein Digest Medium)

AOAC BAM BS12 CCAM CLSI CMPH2 COMPF EP EPA ISO JP MCM9 SMD USDA USP

Cat. No.	211824	Dehydrated – 100 g [†]
	211825	Dehydrated – 500 g [†]
	211822	Dehydrated – 2 kg [†]
	211823	Dehydrated – 10 kg [†]
	290612	Prepared Bottles (wide mouth), 90 mL – Pkg. of 10 [†]
	290613	Prepared Bottles (wide mouth), 100 mL – Pkg. of 10 [†]
	257213	Sterile Pack Bottles (double bagged), 100 mL – Pkg. of 10

Europe

Cat. No.	257423	Prepared Tubes, 13 mL – Pkg. of 25 [†]
	254960	Prepared Bottles (double-strength), 50 mL – Pkg. of 25
	257248	Prepared Bottles, 100 mL – Pkg. of 10 [†]
	257265	Prepared Bottles (double bagged), 100 mL – Pkg. of 10 [†]
	257276	Prepared Bottles, 100 mL (screw cap) – Pkg. of 25 [†]
	257247	Prepared Bottles, 100 mL (tear off seal with stopper) – Pkg. of 25 [†]
	257307	Prepared Bottles (ETO), 100 mL – Pkg. of 44 [†]
	257316	Prepared Bottles (wide mouth), 150 mL – Pkg. of 25 [†]
	257412	Prepared Bottles, 300 mL – Pkg. of 10 [†]
	257413	Prepared Bottles, 500 mL – Pkg. of 4 [†]
	257414	Prepared Bottles, 600 mL – Pkg. of 4 [†]
	257291	Prepared Bottles (double bagged), 800 mL – Pkg. of 4 [†]

BBL™ Trypticase™ Soy Broth (Soybean-Casein Digest Broth)

AOAC	BAM	BS12	CCAM	CLSI	CMPH2	COMPF	EP	EPA	ISO
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JP	MCM9	SMD	USDA	USP
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Cat. No.	211768	Dehydrated – 500 g [†]
	296264	Sterile, Dehydrated – 500 g
	211771	Dehydrated – 5 lb (2.3 kg) [†]
	211772	Dehydrated – 25 lb (11.3 kg) [†]
	295634	Prepared Tubes, 1 mL (K Tubes) – Ctn. of 100
	221815	Prepared Tubes, 2 mL (K Tubes) – Ctn. of 100
	221715	Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10
	221716	Prepared Tubes, 5 mL (K Tubes) – Ctn. of 100
	221092	Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10
	221093	Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100
	299936	Prepared Tubes, 10 mL (D Tubes) – Ctn. of 100 [†]
	221823	Prepared Tubes, 15 mL (A Tubes) – Ctn. of 100
	299749	Prepared Tubes, 20 mL (A Tubes) – Ctn. of 100 [†]
	297811	Prepared Tubes, 21 mL (A Tubes) – Pkg. of 10
	297380	Prepared Bottles, 30 mL – Each
	299107	Prepared Bottles, 100 mL (serum bottle) – Pkg. of 10 [†]
	299416	Prepared Bottles, 100 mL (septum screw cap) – Pkg. of 10 [†]
	257411	Prepared Bottles, 200 mL (flip cap with stopper) – Pkg. of 10
	299113	Prepared Bottles, 500 mL – Pkg. of 10 [†]

Mexico

Cat. No.	252605	Prepared Tubes, 10 mL
	252736	Prepared Tubes, 5 mL

BBL™ Trypticase™ Soy Broth with 6.5% Sodium Chloride

Cat. No.	211351	Prepared Tubes (K Tubes) – Ctn. of 100
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BBL™ Trypticase™ Soy Broth with Fildes Enrichment

Cat. No.	221403	Prepared Tubes (K Tubes) – Pkg. of 10*
	221404	Prepared Tubes (K Tubes) – Ctn. of 100*

Bacto™ Tryptic Soy Broth without Dextrose**BAM**

Cat. No.	286220	Dehydrated – 500 g
	286210	Dehydrated – 10 kg

*Store at 2-8°C.

†QC testing performed according to USPIEPIJP performance specifications.