

Tryptic Soy Agar/Trypticase™ Soy Agar (Soybean-Casein Digest Agar)

Intended Use

Tryptic Soy Agar and Trypticase™ Soy Agar conform with specifications of *The United States Pharmacopeia (USP)*.

Tryptic (Trypticase) Soy Agar (TSA) is used for the isolation and cultivation of nonfastidious and fastidious microorganisms. It is not the medium of choice for anaerobes.

The 150 × 15 mm-style plates of Trypticase Soy Agar are convenient for use with Taxo™ factor strips in the isolation and differentiation of *Haemophilus* species.

Sterile Pack and Isolator Pack plates are useful for monitoring surfaces and air in clean rooms, Isolator Systems and other environmentally-controlled areas when sterility of the medium is of importance.

Hycheck™ hygiene contact slides are used for assessing the microbiological contamination of surfaces and fluids.

Summary and Explanation

The nutritional composition of TSA has made it a popular medium for many years. It is the medium specified as Soybean-Casein Digest Agar Medium in the *USP* for the total aerobic microbial count portion of the microbial limit testing procedures.¹ The medium is used for a multitude of purposes including maintenance of stock cultures, plate counting, isolation of microorganisms from a variety of specimen types and as a base for media containing blood.²⁻⁴ It is included in the compendia of methods for the examination of water, wastewater and foods,^{5, 6} in the *Bacteriological Analytical Manual*⁷ and is used for testing bacterial contaminants in cosmetics.⁸

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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

Difco™ Tryptic Soy Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent.
Prepared Appearance:	Plain – Light amber, slightly opalescent. With 5% sheep blood – Bright red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Tryptic Soy Agar

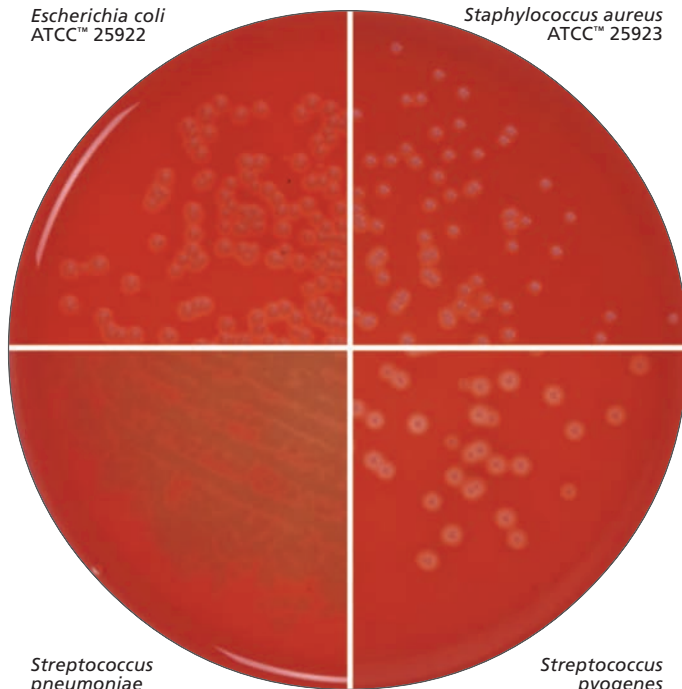
Prepare the medium per label directions, without (plain) and with 5% sheep blood (SB). Inoculate and incubate at 35 ± 2°C for 18-48 hours under 5-10% CO₂.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB	HEMOLYSIS
<i>Escherichia coli</i>	25922	10 ² -10 ³	Good	Good	Beta
<i>Neisseria meningitidis</i>	13090	10 ² -10 ³	Good	Good	None
<i>Staphylococcus aureus</i>	25923	10 ² -10 ³	Good	Good	Beta
<i>Streptococcus pneumoniae</i>	6305	10 ² -10 ³	Good	Good	Alpha
<i>Streptococcus pyogenes</i>	19615	10 ² -10 ³	Good	Good	Beta

CAMP Test medium with 5% sheep blood – Perform using *S. aureus* ATCC 33862, *Streptococcus* sp. group B ATCC 12386 (positive) and *S. pyogenes* ATCC 19615 (negative).

Escherichia coli
ATCC™ 25922

Staphylococcus aureus
ATCC™ 25923



Streptococcus pneumoniae
ATCC™ 6305

Streptococcus pyogenes
ATCC™ 19615

Identity Specifications

BBL™ Trypticase™ Soy Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Plain – Light to medium, yellow to tan, clear to slightly hazy. With 5% sheep blood – Bright red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Trypticase™ Soy Agar

Prepare the medium per label directions, without (plain) and with 5% sheep blood (SB). Inoculate and incubate at 35 ± 2°C for 2 days (incubate streptococci with 3-5% CO₂).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY WITH SB	HEMOLYSIS
<i>Candida albicans</i>	10231	10 ³ -10 ⁴	N/A	Good	None
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	N/A	Good	Beta
<i>Listeria monocytogenes</i>	19115	10 ³ -10 ⁴	N/A	Good	Beta (+/-)
<i>Pseudomonas aeruginosa</i>	10145	10 ³ -10 ⁴	Good	N/A	
<i>Shigella flexneri</i>	12022	10 ³ -10 ⁴	Good	N/A	
<i>Staphylococcus aureus</i>	25923	10 ³ -10 ⁴	Good	Good	Beta
<i>Streptococcus pneumoniae</i>	6305	10 ³ -10 ⁴	Good	Good	Alpha
<i>Streptococcus pyogenes</i>	19615	10 ³ -10 ⁴	Good	Good	Beta

CAMP Test medium with 5% sheep blood – Perform using *S. aureus* ATCC 25923, *Streptococcus* sp. group B ATCC 12386 (positive) and *S. pyogenes* ATCC 19615 (negative).

Since TSA does not contain the X and V growth factors, it can conveniently be used in determining the requirements for these growth factors by isolates of *Haemophilus* by the addition of X, V and XV Factor Strips to inoculated TSA plates.³ The 150 mm plate provides a larger surface area for inoculation, making the “satellite” growth around the strips easier to read.

With the Sterile Pack and Isolator Pack plates, the entire double-wrapped (Sterile Pack) or triple-wrapped (Isolator Pack) product is subjected to a sterilizing dose of gamma radiation, so that the contents inside the outer package(s) are sterile.⁹ This allows the inner package to be aseptically removed without introducing contaminants. Since the agar medium has been sterilized after packaging, the presence of microbial growth after sampling and incubation can be relied upon to represent true recovery and not pre-existing medium contaminants. A third rolled sterile bag is included as a transport device. Isolator Pack plates have been validated to protect the medium from vaporized hydrogen peroxide when used in an Isolator System.

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The Hycheck hygiene contact slide is a double-sided paddle containing two agar surfaces for immersing into fluids or sampling surfaces. There are three slides containing TSA along with another medium: D/E Neutralizing Agar; Violet Red Bile Glucose Agar; or Rose Bengal Chloramphenicol Agar. A fourth slide contains TSA with 0.01% TTC and Rose Bengal Chloramphenicol Agar.

Principles of the Procedure

The combination of casein and soy peptones in TSA renders the medium highly nutritious by supplying organic nitrogen, particularly amino acids and longer-chained peptides. The sodium chloride maintains osmotic equilibrium. Agar is the solidifying agent.

Haemophilus species may be differentiated by their requirements for X and V factors. Paper strips impregnated with these factors are placed on the surface of the medium after inoculation with the test organism. Following incubation, a zone of growth around the strip indicates a requirement for the factor(s).

Formulae

Difco™ Tryptic Soy Agar

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	15.0 g
Enzymatic Digest of Soybean Meal	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g

BBL™ Trypticase™ Soy Agar

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	15.0 g
Papaic Digest of Soybean Meal	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g

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

Directions for Preparation from Dehydrated Product

1. Suspend 40 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. DO NOT OVER-HEAT.
4. For preparation of blood plates, add 5-10% sterile, defibrinated blood to the sterile agar which has been cooled to 45-50°C.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO₂. Incubate plates at 35 ± 2°C for 18-24 hours.

Trypticase™ Soy Agar (150 mm plates) for *Haemophilus*

The initial specimens should be inoculated onto Chocolate II Agar or another suitable medium and incubated for 18-24 hours in an aerobic atmosphere supplemented with carbon dioxide. Choose one or two well-isolated colonies that resemble *Haemophilus* species and perform a Gram stain to confirm that the isolate is a gram-negative rod or coccobacillus. Suspend 1-2 colonies in 5 mL sterile, purified water or Trypticase Soy Broth and vortex to mix. Dip a swab in the suspension and inoculate the entire surface of the plate with the swab. With sterile forceps, place a Taxo X factor strip, a V factor strip and a XV strip on the plate, at least 20 mm apart.

Incubate plates at 35 ± 2°C for 24 hours in an aerobic atmosphere supplemented with carbon dioxide.

Expected Results

After incubation, it is desirable to have isolated colonies of organisms from the original sample. Subculture colonies of interest so that positive identification can be made by means of biochemical and/or serological testing.^{3,10,11}

Consult appropriate texts for the growth patterns produced by the various strains of *Haemophilus*.^{3,12}

References

1. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
3. Forbes, Sahn and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby Inc., St. Louis, Mo.
4. Nash and Krenz. 1991. In Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
5. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
8. Curry, Joyce and McEwen. 1993. CTEA microbiology guidelines. The Cosmetic, Toiletry and Fragrance Association, Inc., Washington, D.C.
9. Association for the Advancement of Medical Instrumentation. 1984. Process control guidelines for gamma radiation sterilization of medical devices. AAMI, Arlington, Va.
10. Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
11. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
12. Campos. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Tryptic Soy Agar (Soybean-Casein Digest Agar)

	AOAC	BAM	CCAM	COMP	EP	ISO	SMD	SMWW	USDA	USP
Cat. No.	236940									
	236950									
	236920									
	236930									

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

	AOAC	BAM	CCAM	COMP	EP	ISO	SMD	SMWW	USDA	USP
Cat. No.	211043									
	211046									
	211047									

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

AOAC	BAM	CCAM	COMPF	EP	ISO	SMD	SMWW	USDA	USP
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United States and Canada

Cat. No.	221185	Prepared Plates – Pkg. of 20*
	221283	Prepared Plates – Ctn. of 100*
	221803	Prepared Plates (150 × 15 mm-style) – Pkg. of 24*
	221082	Prepared Pour Tubes, 20 mL – Pkg. of 10
	221086	Prepared Tubes (K Tubes) – Pkg. of 10
	221087	Prepared Tubes (K Tubes) – Ctn. of 100
	299099	Prepared Bottles, 500 mL – Pkg. of 10

Europe

Cat. No.	254051	Prepared Plates – Pkg. of 20*
	254086	Prepared Plates – Ctn. of 120*
	25007	Prepared Contact Plates – Pkg. of 33*

Japan

Cat. No.	251167	Prepared Plates (5 × 4) – Pkg. of 20*
	251185	Prepared Plates – Pkg. of 20*
	251540	Prepared Plates – Pkg. of 20*
	251260	Prepared Plates (150 × 15 mm-style) – Pkg. of 24*
	251812	Prepared Plates (60 × 15 mm-style) – Ctn. of 240*
	251355	Prepared I Plate™ Dishes – Pkg. of 20*

BBL™ Trypticase™ Soy Agar, Sterile Pack

Cat. No.	221236	Prepared Settling Plates – Pkg. of 10*
	222205	Prepared Settling Plates – Ctn. of 100*
	221237	Prepared Settling Plates (150 × 15 mm-style) – Pkg. of 5*
	222206	Prepared Settling Plates (150 × 15 mm-style) – Ctn. of 45*
	292257	Prepared Plates, heavy fill – Pkg. of 10*
	292396	Prepared Plates, heavy fill – Ctn. of 100*

BBL™ Trypticase™ Soy Agar, Isolator Pack

Cat. No.	292651	Prepared Plates – Pkg. of 10*
	292652	Prepared Plates – Ctn. of 100*
	292272	Prepared Plates (150 × 15 mm-style) – Pkg. of 5*

Difco™ Hycheck™ Hygiene Contact Slides

Cat. No.	290391	Tryptic Soy Agar/D/E Neutralizing Agar (20 slides)*
	290371	Tryptic Soy Agar/Violet Red Bile Glucose Agar (20 slides)*
	290381	Tryptic Soy Agar/Rose Bengal Chloramphenicol Agar (20 slides)*
	290461	Tryptic Soy Agar with 0.01% TTC/Rose Bengal Chloramphenicol Agar (20 slides)*

*Store at 2-8°C.

Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar) • Trypticase™ Soy Agar with Lecithin and Polysorbate 80 • Trypticase™ Soy Agar with Penicillinase • Trypticase™ Soy Agar with Lecithin, Polysorbate 80 and Penicillinase

Intended Use

These media are recommended for the detection and enumeration of microorganisms present on surfaces of sanitary importance. Prepared plates are provided for environmental monitoring. Sterile Pack and Isolator Pack **RODAC™** prepared plates are particularly useful for monitoring surfaces in clean rooms, Isolator Systems and other environmentally-controlled areas and are also recommended for use in air sampling equipment such as the Surface Air System. **Finger Dab™** Sterile Pack and Isolator Pack plates are intended for sampling gloved hands.

Summary and Explanation

These media may be employed to establish and monitor cleaning techniques and schedules.¹⁻⁴ Collection of “samples” from identical areas before and after treatment with disinfectant yields data useful in evaluating cleaning procedures in environmental sanitation. Tryptic (**Trypticase**) Soy Agar with Lecithin and Polysorbate 80 is recommended for the Aerobic Plate Count (Microbial Limit Test) for water-miscible cosmetic products containing preservatives.⁵

RODAC (Replicate Organism Detection and Counting) and contact plates are used in a wide variety of surface sampling programs and may be employed to establish and monitor cleaning techniques and schedules.^{1-4,6} The presence and number of microorganisms on a flat impervious surface is

determined by the appearance of colonies on the surface of the medium following application to the test surface and incubation.^{7,8} The **RODAC** plate has a marked grid to facilitate counting organisms.

The 100 × 15 mm and the 150 × 15 mm style plates can be used for active and passive air sampling. These plates are also designed for personnel monitoring of finger tips (**Finger Dab**).

Principles of the Procedure

Casein and soy peptones are a source of nutrients required for the replication of microorganisms. Sodium chloride maintains osmotic equilibrium. Lecithin and polysorbate 80, two commonly used neutralizers, are reported to inactivate residual disinfectants when the sample is being collected.⁷ Lecithin is incorporated to neutralize quaternary ammonium compounds and polysorbate 80 is used to neutralize substituted phenolic disinfectants.⁹⁻¹² Agar is the solidifying agent.

Trypticase Soy Agar with Penicillinase and **Trypticase** Soy Agar with Lecithin, Polysorbate 80 and Penicillinase contain 50 mL/L of penicillinase, which inactivates antibiotics such as penicillins and cephalosporins.

With the Sterile Pack and Isolator Pack plates, the entire double-wrapped (Sterile Pack) or triple-wrapped (Isolator Pack) product is subjected to a sterilizing dose of gamma radiation,

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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

Difco™ Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

Dehydrated Appearance: Beige, free-flowing, homogeneous, may appear moist.

Solution: 4.57% solution, soluble in purified water upon boiling with frequent gentle swirling. When hot, solution is medium amber, slightly opalescent with a resuspendable precipitate.

Prepared Appearance: Light to medium amber, slightly opalescent, may have a precipitate.

Reaction of 4.57% Solution at 25°C: pH 7.3 ± 0.2

Cultural Response

Difco™ Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

Prepare the medium per label directions. Test the medium in parallel with Plate Count Agar, using the pour plate method. Apply disks impregnated with varying dilutions of a quaternary ammonium compound to the medium surface. Incubate plates at 35 ± 2°C for 40-48 hours and inspect for zones of inhibition.

ORGANISM	ATCC™	INOCULUM CFU	GROWTH*
<i>Escherichia coli</i>	11229	10 ² -10 ³	Smaller zone of inhibition of growth compared to Plate Count Agar
<i>Staphylococcus aureus</i>	6538P	10 ² -10 ³	Smaller zone of inhibition of growth compared to Plate Count Agar

*Interpretation: The smaller zones of inhibition indicate neutralization of the quaternary ammonium compound by the medium.

Identity Specifications

BBL™ Trypticase™ Soy Agar with Lecithin and Polysorbate 80

Dehydrated Appearance: Medium fine, softly lumped powder, free of extraneous material.

Solution: 4.57% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, slightly to moderately hazy.

Prepared Appearance: Light to medium, yellow to tan, slightly to moderately hazy.

Reaction of 4.57% Solution at 25°C: pH 7.3 ± 0.2

Cultural Response

BBL™ Trypticase™ Soy Agar with Lecithin and Polysorbate 80

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 2 days (incubate *A. niger* at 25 ± 2°C for 4 days).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Aspergillus niger</i>	16404	Undiluted	Good
<i>Bacillus subtilis</i>	6633	10 ³ -10 ⁴	Good
<i>Candida albicans</i>	001	10 ³ -10 ⁴	Good
<i>Enterococcus faecalis</i>	29212	10 ³ -10 ⁴	Good
<i>Micrococcus luteus</i>	9341	10 ³ -10 ⁴	Good
<i>Pseudomonas aeruginosa</i>	10145	10 ³ -10 ⁴	Good
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	13311	10 ³ -10 ⁴	Good
<i>Serratia marcescens</i>	13880	10 ³ -10 ⁴	Good
<i>Staphylococcus aureus</i>	25923	10 ³ -10 ⁴	Good
<i>Staphylococcus epidermidis</i>	12228	10 ³ -10 ⁴	Good
<i>Streptococcus pyogenes</i>	19615	10 ³ -10 ⁴	Good

so that the contents inside the outer package(s) are sterile.¹³ This allows the inner package to be aseptically removed without introducing contaminants. Since the agar medium has been sterilized after packaging, the presence of microbial growth after sampling and incubation can be relied upon to represent true recovery and not pre-existing medium contaminants. A third rolled sterile bag is included as a transport device. Isolator Pack plates have been validated to protect the medium from vaporized hydrogen peroxide when used in an Isolator System.

Formulae

Difco™ Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	15.0 g
Soy Peptone	5.0 g
Sodium Chloride	5.0 g
Lecithin	0.7 g
Polysorbate 80	5.0 g
Agar	15.0 g

BBL™ Trypticase™ Soy Agar with Lecithin and Polysorbate 80

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	15.0 g
Papaic Digest of Soybean Meal	5.0 g
Sodium Chloride	5.0 g
Lecithin	0.7 g
Polysorbate 80	5.0 g
Agar	15.0 g

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

Directions for Preparation from Dehydrated Product

1. Suspend 45.7 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to approximately 45°C.

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- In RODAC plates, use 16.5-17.5 mL per plate.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

100 × 15 mm and 150 × 15 mm-Style Plates

- If specimen is being cultured from a swab, roll the swab directly on the medium surface.
- Incubate all plates at 35-37°C for 48 hours, and 25°C for 7 days or as required.
- When incubation has been completed, count the colonies.

RODAC™/Contact Plates

Selected surfaces are sampled by firmly pressing the agar medium against the test area. Hold the plate with thumb and second finger and use index finger to press plate bottom firmly against surface. Pressure should be the same for every sample. Do not move plate laterally; this spreads contaminants over the agar surface making resolution of colonies difficult. Slightly curved surfaces may be sampled with a rolling motion.

Areas (walls, floors, etc.) to be assayed may be divided into sections or grids and samples taken from specific points within the grid.

Grid method:

- Subdivide surface (floor or wall) into 36 equal squares per 100 square feet of area by striking five equidistant dividing lines from each of the two adjacent sides.
- These dividing lines intersect at twenty-five points.
- Number these intersections consecutively in a serpentine configuration.
- Use red numerals for odd numbers, black numerals for even numbers.
- Omit number 13 which falls in the center of the total area.
- Sample odd points at one sampling period, even points at the next sampling period.
- For areas greater than 100 square feet, extend grid to include entire area.
- For areas smaller than 25 square feet, divide the areas into twenty-five equal squares (sixteen intersections). Sample eight even-numbered or odd-numbered intersections at each sampling period.
- For areas between 25 and 100 square feet, divide into 36 equal squares as in #1.
- Mark plates with intersection numbers.

Incubate exposed plates at 35-37°C for 48 hours, and 25°C for 7 days or as required.

Expected Results

Because interpretations are relative, each laboratory should establish its own values for what constitutes a clean area.

Count all developing colonies. Spreading colonies should be counted as one but care should be taken to observe other distinct colonies intermingled in the growth around the plate periphery or along a hair line. These should also be counted as one colony, as should bi-colored colonies and halo type spreaders.

It is generally agreed that 200 colonies is the approximate maximum that can be counted on contact plates.

Colony counts may be recorded by:

- Simply keeping individual counts.
- Number of viable particles per square foot (agar area is 3.97 square inches).
- Means and standard deviations.

Subculture colonies of interest so that positive identification can be made by means of biochemical and/or serological testing.

Limitation of the Procedure

The effectiveness of preservative neutralization with this medium depends on both the type and concentration of the preservative(s).

References

- Vesley and Michaelson. 1964. *Health Lab. Sci.* 1:107.
- Pryor and McDuff. 1969. *Exec. Housekeeper*, March.
- Dell, L. A. 1979. *Pharm. Technol.* 3:47.
- Hickey, Beckelheimer and Parrow. 1993. *In* Marshall (ed.), *Standard methods for the examination of dairy products*, 16th ed. American Public Health Association, Washington, D.C.
- Orth. 1993. *Handbook of cosmetic microbiology*. Marcel Dekker, Inc., New York, N.Y.
- Hall and Hartnett. 1964. *Public Health Rep.* 79:1021.
- McGowan. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.
- Bryan. 1995. *In* Murray, Baron, Pfaller, Tenover and Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
- Favero, Gabis and Vesley. 1984. *In* Speck (ed.), *Compendium of methods for the microbiological examination of foods*, 2nd ed. American Public Health Association, Washington, D.C.
- Quisno, Gibby and Foter. 1946. *Am. J. Pharm.* 118:320.
- Erlanson and Lawrence. 1953. *Science* 118:274.
- Sveum, Moberg, Rude and Frank. 1992. *In* Vanderzant and Splittstoesser (ed.), *Compendium of methods for the examination of foods*, 3rd ed. American Public Health Association, Washington, D.C.
- Association for the Advancement of Medical Instrumentation. 1984. *Process control guidelines for gamma radiation sterilization of medical devices*. AAMI, Arlington, Va.

Availability

Difco™ Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

Cat. No. 255320 Dehydrated – 500 g*
255310 Dehydrated – 2 kg*

BBL™ Trypticase™ Soy Agar with Lecithin and Polysorbate 80

Cat. No. 211764 Dehydrated – 500 g*
212263 Dehydrated – 5 lb (2.3 kg)*
296033 Dehydrated – 25 lb (11.3 kg)*

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United States and Canada

Cat. No.	221943	Prepared Plates (Double Bag) – Ctn. of 100*
	221945	Contact Plates (Double Bag) – Pkg. of 20*
	221288	Prepared RODAC ™ Plates – Pkg. of 10*
	221287	Prepared RODAC ™ Plates – Ctn. of 100*
	221961	Sterile Pack Contact Plates – Pkg. of 10*
	222208	Sterile Pack Contact Plates – Ctn. of 100*
	221238	Sterile Pack RODAC ™ Plates – Pkg. of 10*
	222207	Sterile Pack RODAC ™ Plates – Ctn. of 100*
	299896	Isolator Pack RODAC ™ Plates – Pkg. of 10*
	292335	Isolator Pack RODAC ™ Plates – Ctn. of 100*
	292273	Sterile Pack Finger Dab ™ Plates – Pkg. of 10*
	292271	Sterile Pack Finger Dab ™ Plates – Ctn. of 100*
	292305	Sterile Pack Finger Dab ™ Plates (150 × 15 mm-style) – Pkg. of 5*
	292648	Isolator Pack Finger Dab ™ Plates – Pkg. of 10*
	292649	Isolator Pack Finger Dab ™ Plates – Ctn. of 100*
	292650	Isolator Pack Finger Dab ™ Plates (150 × 15 mm-style) – Pkg. of 5*

Europe

Cat. No. 254038 Contact Plates – Pkg. of 33*

BBL™ Trypticase™ Soy Agar with Penicillinase, Sterile Pack

Cat. No.	221839	Prepared Plates – Pkg. of 10*
	221837	Prepared Plates (150 × 15 mm-style) – Pkg. of 5*
	292256	Prepared Plates (Deep Fill) – Pkg. of 10*

BBL™ Trypticase™ Soy Agar with Lecithin, Polysorbate 80 and Penicillinase, Sterile Pack

Cat. No.	221987	Contact Plates – Pkg. of 10*
	221234	Prepared RODAC ™ Plates – Pkg. of 10*
	292301	Prepared Finger Dab ™ Plates – Ctn. of 100*

*Store at 2-8°C.

TSA Blood Agars

Tryptic Soy Blood Agar Base No. 2 • Tryptic Soy Blood Agar Base EH • Trypticase™ Soy Agar, Modified (TSA II) Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) Trypticase™ Soy Agar with 10% Sheep Blood (TSA II) Trypticase™ Soy Agar with 5% Horse Blood (TSA II) Trypticase™ Soy Agar with 5% Rabbit Blood (TSA II)

Intended Use

Tryptic Soy Blood Agar Base No. 2, Tryptic Soy Blood Agar Base EH and **Trypticase** Soy Agar, Modified (TSA II) supplemented with blood are used for cultivating fastidious microorganisms and for the visualization of hemolytic reactions produced by many bacterial species.

Summary and Explanation

The nutritional composition of TSA (Tryptic Soy Agar/ **Trypticase** Soy Agar) has made it a popular medium, both unsupplemented and as a base for media containing blood. **Trypticase** Soy Agar, Modified (TSA II) is an improved version of the original TSA formulation for use with animal blood supplements. With 5 or 10% sheep blood, it is extensively used for the recovery and cultivation of fastidious microbial species and for the determination of hemolytic reactions that are important differentiating characteristics for bacteria, especially *Streptococcus* species. Some investigators prefer the use of rabbit or horse blood, but **Trypticase** Soy Agar with 5% Horse Blood is not recommended for use with throat cultures.

Tryptic Soy Blood Agar Base No. 2 and Tryptic Soy Blood Agar Base EH (enhanced hemolysis) are additional options when hemolytic reactions are important. Tryptic Soy Blood Agar Base No. 2 provides clear hemolytic reactions with group A streptococci, while Tryptic Soy Blood Agar Base EH provides dramatic, improved hemolysis.

Blood agar base media are specified in standard methods for food testing.^{1,2}

Principles of the Procedure

The combination of casein and soy peptones in **Trypticase** Soy Agar, Modified (TSA II) renders the medium highly nutritious by supplying organic nitrogen, particularly amino acids and larger-chained peptides. The sodium chloride maintains osmotic equilibrium. Agar is the solidifying agent.

Tryptic Soy Blood Agar Base No. 2 and Tryptic Soy Blood Agar Base EH are similar in composition to TSA II. These formulations utilize the peptones Tryptone H and Tryptone H Plus to enhance hemolysin production while minimizing antagonism or loss in activity of streptococcal hemolysins.

Defibrinated sheep blood is the most widely used blood for enriching agar base media.³ Hemolytic reactions of streptococci are proper and growth of *Haemophilus hemolyticus*, a nonpathogen whose hemolytic colonies are indistinguishable from those of beta-hemolytic streptococci, is inhibited.

Trypticase Soy Agar with 5% Sheep Blood (TSA II) prepared plates provide excellent growth and beta hemolysis by *Streptococcus pyogenes* (Lancefield group A) and also provide excellent growth and appropriate hemolytic reactions with other fastidious organisms. This medium is suitable for performing the CAMP test for the presumptive identification

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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

Difco™ Tryptic Soy Blood Agar Base No. 2 or Tryptic Soy Blood Agar Base EH

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to slightly opalescent.
Prepared Appearance:	Plain – Light to medium amber, clear to slightly opalescent. With 5% sheep blood – Bright cherry red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Tryptic Soy Blood Agar Base No. 2* or Tryptic Soy Blood Agar Base EH

Prepare the medium per label directions with 5% sheep blood. Inoculate and incubate at 35 ± 2°C for 18-48 hours under 5-10% CO₂.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS TSA NO. 2	HEMOLYSIS TSA EH
<i>Escherichia coli</i>	25922	10 ² -10 ³	Good	Beta	Beta
<i>Neisseria meningitidis</i>	13090	10 ² -10 ³	Good	None	None
<i>Staphylococcus aureus</i>	25923	10 ² -10 ³	Good	Beta	Beta
<i>Streptococcus pneumoniae</i>	6305	10 ² -10 ³	Good	Alpha	Alpha
<i>Streptococcus pyogenes</i>	19615	10 ² -10 ³	Good	Beta	Beta

*CAMP Test – Perform using *S. aureus* ATCC 25923, *Streptococcus sp. group B* ATCC 12386 (positive) and *S. pyogenes* ATCC 19615 (negative).

Identity Specifications

BBL™ Trypticase™ Soy Agar, Modified (TSA II)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.0% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Plain – Light to medium, yellow to tan, clear to slightly hazy. With 5% sheep blood – Bright red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ Trypticase™ Soy Agar, Modified (TSA II)

Prepare the medium per label directions with 5% sheep blood. Inoculate and incubate at 35 ± 2°C for 18-48 hours (incubate streptococci with 3-5% CO₂).

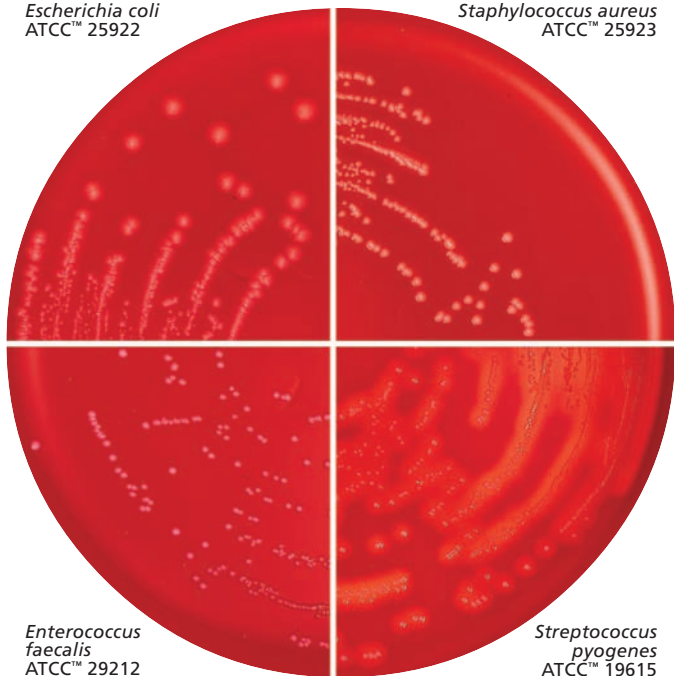
ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	HEMOLYSIS
<i>Candida albicans</i>	10231	10 ³ -10 ⁴	Good	None
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	Good, within 24 hours	Beta
<i>Listeria monocytogenes</i>	19115	10 ³ -10 ⁴	Good	Beta (+/-)
<i>Shigella dysenteriae</i>	9361	10 ³ -10 ⁴	Good	None
<i>Staphylococcus aureus</i>	25923	10 ³ -10 ⁴	Good, within 24 hours	Beta
<i>Staphylococcus aureus</i>	6538P	10 ³ -10 ⁴	Good	Beta
<i>Streptococcus pneumoniae</i>	6305	10 ³ -10 ⁴	Good, within 24 hours	Alpha
<i>Streptococcus pyogenes</i>	19615	10 ³ -10 ⁴	Good, within 24 hours	Beta
<i>Streptococcus pyogenes</i>	49117	10 ³ -10 ⁴	Good	Beta

CAMP Test – Perform using *S. aureus* ATCC 25923, *Streptococcus sp. group B* ATCC 12386 (positive) and *S. pyogenes* ATCC 19615 (negative).

TSA II with 5% Sheep Blood

Escherichia coli
ATCC™ 25922

Staphylococcus aureus
ATCC™ 25923



Enterococcus faecalis
ATCC™ 29212

Streptococcus pyogenes
ATCC™ 19615

of group B streptococci (*S. agalactiae*), and for use with low concentration (0.04 unit) bacitracin discs (**Taxo™ A**) for presumptive identification of group A streptococci (*S. pyogenes*).

The CAMP test is based on the formation of a zone of synergistic hemolysis at the junction of perpendicular streak inocula of *Staphylococcus aureus* and group B streptococci. The reaction is caused by the sphingomyelinase (beta-toxin) of *S. aureus* reacting with sphingomyelin in the sheep erythrocyte membrane to produce ceramide. A non-enzymatic protein (CAMP protein), produced by *S. agalactiae*, binds to the ceramide and leads to disorganization of the lipid bilayer of the sheep erythrocyte membrane resulting in complete lysis.⁴

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Trypticase Soy Agar with 10% Sheep Blood (TSA II) prepared plates are provided for those laboratories preferring the increased blood content. This medium is not recommended for performance of the CAMP test. Additionally, the increased blood content can make hemolytic reactions less distinct and more difficult to read.

Trypticase Soy Agar with 5% Horse Blood (TSA II) prepared plates supply both the X and V factors which are growth requirements for certain organisms; e.g., *Haemophilus influenzae*. Sheep and human blood are not suitable for this purpose because they contain enzymes that inactivate the nicotinamide adenine dinucleotide (NAD), which is the V factor.⁵

Defibrinated horse blood may give hemolytic reactions different from sheep blood.³ Some streptococci (e.g., group D) give hemolytic reactions on horse blood, but not on sheep blood and may be mistakenly reported as group A. If a hemolytic reaction is obtained, the organisms should be tested with a **Taxo A** disc and grouped serologically or tested by the fluorescent method.⁶ Beta-hemolytic streptococci and *Haemophilus hemolyticus* may be differentiated by performing a Gram stain on a smear prepared from the colony.⁷

Defibrinated rabbit blood is also used for enriching agar-based media.⁸ Hemolytic reactions on **Trypticase Soy Agar with 5% Rabbit Blood (TSA II)** prepared plates are similar to those on sheep blood. However, rabbit blood does not inhibit *Haemophilus haemolyticus*, a bacterium inhibited by sheep blood that produces colonies indistinguishable from those of beta-hemolytic streptococci.

Formulae

Difco™ Tryptic Soy Blood Agar Base No. 2

Approximate Formula* Per Liter	
Tryptone H	15.0 g
Soytone	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g

Difco™ Tryptic Soy Blood Agar Base EH

Approximate Formula* Per Liter	
Tryptone H Plus	15.0 g
Soytone	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g

BBL™ Trypticase™ Soy Agar, Modified (TSA II)

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	14.5 g
Papaic Digest of Soybean Meal	5.0 g
Sodium Chloride	5.0 g
Agar	14.0 g
Growth Factors	1.5 g

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

Directions for Preparation from Dehydrated Product

1. Suspend 40 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. DO NOT OVER-HEAT.
4. For preparation of blood plates, add 5-10% sterile, defibrinated blood to sterile agar which has been cooled to 45-50°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. After streaking, stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the activity of both oxygen-stable and oxygen-labile streptolysins.⁹

Incubate plates at 35 ± 2°C for 18-72 hours. Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 5% CO₂.

CAMP Test¹⁰

Non-hemolytic, bile-esculin negative streptococci or bacitracin-resistant beta-hemolytic streptococci may be tested by the CAMP test for presumptive identification as *S. agalactiae* (Lancefield group B). The inoculum may be taken from an overnight broth culture or from colonies picked from a blood agar plate. Make a single streak of *Staphylococcus aureus* ATCC 33862 across the center of a blood agar plate. If a loop is used, do not use it parallel to the agar surface, since the streak will be too wide and the results will not be satisfactory. The streptococcal isolates to be tested are inoculated by making a simple streak perpendicular to the *S. aureus* line coming as close as possible (2-3 mm), but not touching it. Several streptococcal isolates may be tested on the same plate. Perpendicular streptococcal streaks should be 5-8 mm apart. Include a known *S. agalactiae* for a positive control and *S. pyogenes* as a negative control. The procedure should be practiced with known cultures before using it to identify unknown isolates.

NOTE: Studies on the CAMP Test have shown that the reaction is most reliable early in the shelf life of some lots of the prepared plated medium. It is recommended that *S. agalactiae* ATCC 12386 be included along with patient isolates to verify satisfactory performance.

Incubate plates in an aerobic atmosphere at 35 ± 2°C for 18-24 hours. Do not incubate anaerobically or in a CO₂ incubator. False-positive results may occur with group A streptococci when incubation is in an anaerobic or CO₂-enriched atmosphere.^{10,11}

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Expected Results

Hemolytic streptococci may appear translucent or opaque, grayish, small (1 mm), or large matt and mucoid (2-4 mm) colonies, encircled by a zone of hemolysis. Gram stains should be made and examined to check the macroscopic findings. (Other organisms which may cause hemolysis include *Listeria*, various corynebacteria, hemolytic staphylococci, *Escherichia coli* and *Pseudomonas*.) In reporting, approximate quantitation of the number of colonies of hemolytic streptococci may be helpful to the clinician.

- Pneumococci usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of “green” (alpha) hemolysis.
- Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.
- *Listeria* produce small zones of beta hemolysis. They may be distinguished by their rod shape in stains, and by motility at room temperature.
- *Haemophilus influenzae* produces nonhemolytic, small grayish, translucent colonies with a “mousy” odor on Trypticase Soy Agar (TSA II) with 5% Rabbit Blood.
- Other organisms representing minimal flora and clinically significant isolates can also be expected to grow on this nonselective formulation.

CAMP Test

A positive CAMP reaction is indicated by an arrowhead or triangular shaped area of increased hemolysis which forms around the end of the streptococcal streak line closest to the *S. aureus* growth. The streptococcal growth must be within the wide zone of partial hemolysis that surrounds the *S. aureus* growth. A negative reaction may appear as a bullet-shaped zone of slightly increased hemolysis or as no increased hemolysis.

Bacitracin-negative, CAMP-positive, beta-hemolytic streptococci may be reported as presumptive group B streptococci. CAMP-positive group A species may be differentiated from group B streptococci by hemolysis, bacitracin susceptibility, and hippurate hydrolysis. Group B streptococci tend to produce larger colonies and have less pronounced zones of beta hemolysis than other beta-hemolytic strains, and some group B strains are nonhemolytic.⁹

References

1. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
2. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. Vera and Power. 1980. In Lennette, Balows, Hausler and Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
4. Bernheimer, Linder and Avigad. 1979. Infect. Immun. 23:838.
5. Krumweide and Kuttner. 1938. J. Exp. Med. 67:429.
6. Vera. 1971. Health Lab Sci. 8:176.
7. Finegold and Martin. 1982. Bailey & Scott's diagnostic microbiology, 6th ed. The C.V. Mosby Company, St. Louis, Mo.
8. Nash and Krenz. 1991. In Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
9. Ruoff, Whiley and Beighton. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
10. Darling. 1975. J. Clin. Microbiol. 1:171.
11. Facklam and Washington. 1991. In Balows, Hausler, Herrmann, Isenberg and Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Tryptic Soy Blood Agar Base No. 2

BAM	COMPF	
Cat. No.	227300	Dehydrated – 500 g
	227200	Dehydrated – 10 kg

Difco™ Tryptic Soy Blood Agar Base EH

BAM	COMPF	
Cat. No.	228300	Dehydrated – 500 g
	228200	Dehydrated – 10 kg

BBL™ Trypticase™ Soy Agar, Modified (TSA II)

BAM	COMPF	
Cat. No.	212305	Dehydrated – 500 g
	298031	Dehydrated, LitrePak™ – 20 x 1.0 L
	221082	Prepared Pour Tubes, 20 mL – Pkg. of 10
	297941	Prepared Pour Tubes, 20 mL – Ctn. of 100

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

BS10	CMPH	MCM7
<i>United States and Canada</i>		
Cat. No.	221239	Prepared Plates – Pkg. of 20*
	221261	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	254053	Prepared Plates – Pkg. of 20*
	254087	Prepared Plates – Ctn. of 120*

Japan

Cat. No.	251239	Prepared Plates – Pkg. of 20*
	251261	Prepared Plates – Ctn. of 100*
	251240	Prepared Plates – Ctn. of 200*

BBL™ Trypticase Soy Agar with 10% Sheep Blood (TSA II)

Cat. No.	221162	Prepared Plates – Pkg. of 20*
	221260	Prepared Plates – Ctn. of 100*

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)// Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

Cat. No.	221292	Prepared I Plate™ Dishes – Pkg. of 20*
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BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//Group A Selective Strep Agar with 5% Sheep Blood (ssA™)

Cat. No.	221783	Prepared Bi-Plate Dishes – Pkg. of 20*
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BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//Chocolate II Agar

BS10	CMPH	MCM7
<i>United States and Canada</i>		
Cat. No.	221302	Prepared I Plate™ Dishes – Pkg. of 20*
	221303	Prepared I Plate™ Dishes – Ctn. of 100*

Europe

Cat. No.	251302	Prepared I Plate™ Dishes – Pkg. of 20*
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Japan

Cat. No.	251302	Prepared I Plate™ Dishes – Pkg. of 20*
	251303	Prepared I Plate™ Dishes – Ctn. of 100*

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//Levine EMB Agar

BS10	CMPH	MCM7
Cat. No.	221286	Prepared I Plate™ Dishes – Pkg. of 20*
	221289	Prepared I Plate™ Dishes – Ctn. of 100*

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TSA II with Ampicillin

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//MacConkey II Agar

BS10 CMPH MCM7

United States and Canada

Cat. No. 221290 Prepared **I Plate™** Dishes – Pkg. of 20*
221291 Prepared **I Plate™** Dishes – Ctn. of 100*

Europe

Cat. No. 251290 Prepared **I Plate™** Dishes – Pkg. of 20*

Japan

Cat. No. 251290 Prepared **I Plate™** Dishes – Ctn. of 20*
251572 Prepared **I Plate™** Dishes – Ctn. of 100*

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//MacConkey II Agar with MUG

Cat. No. 221949 Prepared **I Plate™** Dishes – Pkg. of 20*

BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)//Chocolate II Agar//MacConkey II Agar

Cat. No. 297140 Prepared **Y Plate™** Dishes – Pkg. of 20*
299580 Prepared **Y Plate™** Dishes – Ctn. of 100*

BBL™ Trypticase™ Soy Agar with 5% Horse Blood (TSA II)

United States and Canada

Cat. No. 221372 Prepared Plates – Pkg. of 20*

Europe

Cat. No. 212099 Prepared Plates – Pkg. of 20*

BBL™ Trypticase™ Soy Agar with 5% Rabbit Blood (TSA II)

Cat. No. 221356 Prepared Plates – Pkg. of 20*

BBL™ Trypticase™ Soy Agar (TSA II) with Defibrinated Sheep Blood Slant

Cat. No. 220830 Prepared Slants – Pkg. of 10*
220831 Prepared Slants – Ctn. of 100*

*Store at 2-8°C.

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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 conforms with specifications of *The United States Pharmacopeia (USP)*.

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*.

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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™ 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

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 18-48 hours (up to 72 hours, if necessary). To test for growth promotion according to the USP/EP, inoculate using organisms marked with (*) and incubate at 20-25°C for 3 days and 7 days for bacteria and fungi, respectively (incubate *B. subtilis* at 20-25°C and 30-35°C).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	USP/EP GROWTH
<i>Neisseria meningitidis</i>	13090	10-10 ²	Fair to good	N/A
<i>Staphylococcus epidermidis</i>	12228	10-10 ²	Good	N/A
<i>Streptococcus pneumoniae</i>	6305	10-10 ²	Good	N/A
<i>Streptococcus pyogenes</i>	19615	10-10 ²	Good	N/A
<i>Aspergillus niger</i> *	16404	10-10 ²	N/A	Growth
<i>Bacillus subtilis</i> * (20-25°C)	6633	10-10 ²	N/A	Growth
<i>Bacillus subtilis</i> * (30-35°C)	6633	10-10 ²	N/A	Growth
<i>Candida albicans</i> *	10231	10-10 ²	N/A	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

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

Cultural Response

BBL™ Trypticase™ Soy Broth

Prepare the medium per label directions. Inoculate and incubate aerobically (unless otherwise indicated) at 20-25°C for organisms marked (*) and at 33-37°C for the remaining organisms. Incubate for up to 5 days (incubate *E. coli* and *S. aureus* for 2 days).

ORGANISM	ATCC™	INOCULUM CFU	RESULT
<i>Aspergillus niger</i> *	16404	< 10 ²	Growth
<i>Bacillus subtilis</i> *	6633	< 10 ²	Growth
<i>Bacteroides fragilis</i> (anaerobic)	25285	5 × 10 ² -10 ³	Growth
<i>Candida albicans</i> *	10231	< 10 ²	Growth
<i>Candida albicans</i> *	2091	< 10 ²	Growth
<i>Escherichia coli</i>	25922	< 10 ²	Growth
<i>Micrococcus luteus</i> *	9341	< 10 ²	Growth
<i>Neisseria meningitidis</i> (3-5% CO ₂)	700344	< 10 ²	Growth
<i>Pseudomonas aeruginosa</i>	27853	5 × 10 ² -10 ³	Growth
<i>Staphylococcus aureus</i>	25923	< 10 ²	Growth
<i>Streptococcus pneumoniae</i>	6305	< 10 ²	Growth
<i>Streptococcus pneumoniae</i> (anaerobic)	6305	< 10 ²	Growth

NOTE: *A. niger* ATCC 16404, *B. subtilis* ATCC 6633 and *C. albicans* ATCC 10231 are the USP/EP recommended organisms for growth promotion testing.



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

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.¹⁻⁴ Because of its capacity for growth promotion, this formulation is included in the *USP* as a sterility test medium and for use in performing microbial limit testing.⁵

TSB was chosen by the USDA Animal and Plant Health Inspection Service for detecting viable bacteria in live vaccines.⁶ It is used in the coliphage detection procedure, a methodology in *Standard Methods for the Examination of Water and Wastewater*.⁷ TSB is recommended for testing bacterial contaminants in cosmetics⁸ and complies with established standards in the food industry.⁹⁻¹¹

TSB is also recommended, because of growth promotion, for use as the inoculum broth for disc diffusion and agar dilution antimicrobial susceptibility testing as standardized by the National Committee for Clinical Laboratory Standards (NCCLS).^{12,13}

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.¹⁴

Trypticase Soy Broth supplemented with 5% Fildes Enrichment provides growth factors necessary for the cultivation of fastidious organisms.¹⁵

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 meal 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.

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.¹

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

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
Dextrose	2.5 g

BBL™ Trypticase™ Soy Broth

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	17.0 g
Papaic Digest of Soybean Meal	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

- 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.
- Warm gently until solution is complete.
- Autoclave at 121°C for 15 minutes.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

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 BBL™ GasPak™ or GasPak EZ anaerobic systems or equivalent alternative system.

Examine for growth after 18-24 hours and 42-48 hours of incubation.

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For use in sterility testing, consult the *USP* for procedural details and specifications for volume of medium relative to container size.⁵

For use in the preparation of standardized inocula for antimicrobial susceptibility testing, refer to the NCCLS standards.^{12,13}

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

- MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
- Marshall (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
- Forbes, Sahm and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc. St. Louis, Mo.
- 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.
- United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
- Federal Register. 1992. Fed. Regist. 21:113.26.
- Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
- Curry, Joyce and McEwen. 1993. CTFA microbiology guidelines. The Cosmetic, Toiletry and Fragrance Association, Inc., Washington, D.C.
- U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
- Horwitz (ed.). 2000. Official methods of analysis AOAC International, 17th ed., vol. 1. AOAC International, Gaithersburg, Md.
- Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
- National Committee for Clinical Laboratory Standards. 2000. Approved standard: M2-A7. Performance standards for antimicrobial disk susceptibility tests, 7th ed., NCCLS, Wayne, Pa.
- National Committee for Clinical Laboratory Standards. 2000. Approved standard: M7-A5. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. NCCLS, Wayne, Pa.
- Facklam, Sahm and Teixeira. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
- Fildes. 1920. Br. J. Exp. Pathol. 1:129.

Availability

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

AOAC BAM BS10 CCAM CMPH COMPF EP ISO MCM7
NCCLS SMD SMWW USDA USP

Cat. No.	211824	Dehydrated – 100 g
	211825	Dehydrated – 500 g
	211822	Dehydrated – 2 kg
	211823	Dehydrated – 10 kg
	290631	Prepared Bottles, 100 mL (septum screw cap) – Pkg. of 10
	290612	Prepared Bottles, 90 mL (wide mouth) – Pkg. of 10
	290613	Prepared Bottles, 100 mL (wide mouth) – Pkg. of 10
	257213	Sterile Pack Bottles (double bagged), 100 mL – Pkg. of 10

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

AOAC BAM BS10 CCAM CMPH COMPF EP ISO MCM7
NCCLS SMD SMWW USDA USP

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)
	298131	Dehydrated LitrePak™ – 20 × 1.0 L
	297294	Prepared Tubes, 0.5 mL (K Tubes) – Ctn. of 100
	295634	Prepared Tubes, 1 mL (K Tubes) – Ctn. of 100
	221815	Prepared Tubes, 2 mL (K Tubes) – Ctn. of 100
	297979	Prepared Tubes, 3 mL (K Tubes) – Ctn. of 100
	297482	Prepared Tubes, 4 mL (D Tubes) – Ctn. of 100
	221715	Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10
	221716	Prepared Tubes, 5 mL (K Tubes) – Ctn. of 100
	297342	Prepared Tubes, 5 mL (D Tubes) – Ctn. of 100
	221092	Prepared Tubes, 8 mL (K Tubes) – Pkg. of 10
	221093	Prepared Tubes, 8 mL (K Tubes) – Ctn. of 100
	297354	Prepared Tubes, 10 mL (D Tubes) – Ctn. of 100
	221823	Prepared Tubes, 15 mL (A Tubes) – Ctn. of 100
	297811	Prepared Tubes, 21 mL (A Tubes) – Pkg. of 10
	297380	Prepared Bottle – 30 mL
	299107	Prepared Bottles, 100 mL (serum bottle) – Pkg. of 10
	299416	Prepared Bottles, 100 mL (septum screw cap) – Pkg. of 10
	299113	Prepared Bottles, 500 mL – Pkg. of 10

BBL™ Trypticase™ Soy Broth with 6.5% Sodium Chloride

Cat. No.	221351	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

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

*Store at 2-8°C.

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