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

Intended Use

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.

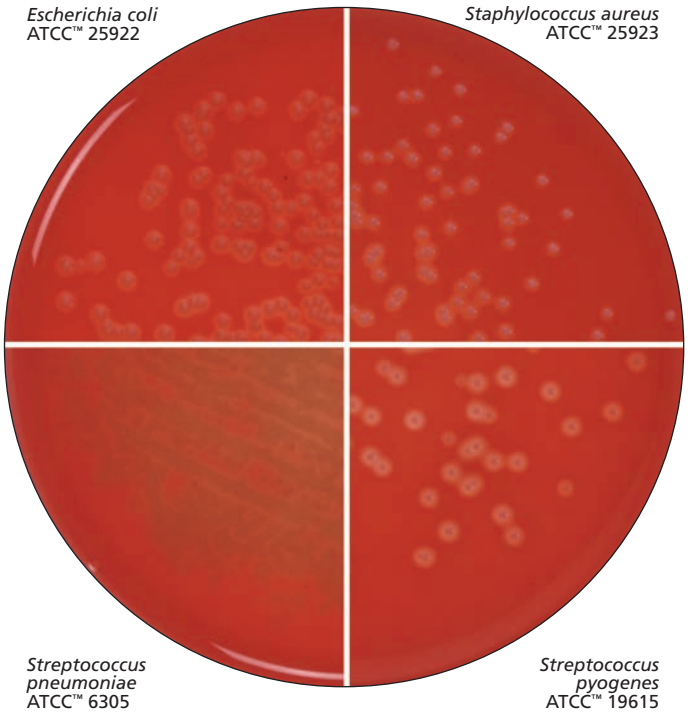
Sterile Pack and Isolator Pack plates are useful for monitoring surfaces and air in clean rooms, Isolator Systems and other environmentally-controlled areas when sterility of the medium is of importance.

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

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

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 General Chapter <61> of the *USP* when performing enumerations tests for nonsterile pharmaceutical products.[1] The medium is used in *USP* Growth Promotion testing and when testing the suitability of counting methods in the presence of product. TSA has a multitude of uses in the clinical laboratory including maintenance of stock cultures, plate counting, isolation of microorganisms from a variety of specimen types and as a base for media containing blood.[4-7] It is also recommended for use in industrial applications when testing water and wastewater,[8] food,[9-14] dairy products,[15] and cosmetics.[10,16]



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.[5] 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.[17] 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.

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
Papaic Digest of Soybean	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	5.0	g
Sodium Chloride	5.0	g
Agar	15.0	g

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

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 with 5-10% CO_2 for 18-48 hours. Incubate (*) cultures at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY W/SB	HEMOLYSIS
Escherichia coli	25922	30-300	Good	Good	Beta
Neisseria meningitidis	13090	30-300	Good	Good	None
Staphylococcus aureus	25923	30-300	Good	Good	Beta
Streptococcus pneumoniae	6305	30-300	Good	Good	Alpha
Streptococcus pyogenes	19615	30-300	Good	Good	Beta
*Aspergillus brasiliensis (niger)**	16404	<100	Growth	N/A	N/A
*Bacillus subtillis**	6633	<100	Growth	N/A	N/A
*Candida albicans**	10231	<100	Growth	N/A	N/A
*Escherichia coli**	8739	<100	Growth	N/A	N/A
*Pseudomonas aeruginosa**	9027	<100	Growth	N/A	N/A
Salmonella enterica subsp. *enterica* serotype Typhimurium*	14028	<100	Growth	N/A	N/A
*Staphylococcus aureus**	6538	<100	Growth	N/A	N/A

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).

Continued

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 OVERHEAT.
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.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.[4-7]

For water, food, dairy or cosmetic samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[8-16]

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 Agar.[4-7]

For water, food, dairy or cosmetic samples, refer to appropriate standard references for details on test methods using Tryptic/**Trypticase** Soy Agar.[8-16]

For pharmaceutical samples, refer to *USP* General Chapter <61> for details on the examination of nonsterile products and performing microbial enumeration tests using Tryptic/**Trypticase** Soy Agar.[1]

Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 3-10% CO_2. 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.

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

BBL™ Trypticase™ Soy Agar (prepared bottle)

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

Reaction at 25°C: pH 7.3 ± 0.2

BBL™ Trypticase™ Soy Agar (prepared plate)

Appearance: Light to medium tan yellow, hazy.

Reaction at 25°C: pH 7.3 ± 0.2

BBL™ Trypticase™ Soy Agar (prepared Sterile Pack plate)

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

Reaction 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 48 hours (incubate *S. pneumoniae* and *S. pyogenes* with 3-5% CO_2). Incubate (*) cultures at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY PLAIN	RECOVERY W/SB	HEMOLYSIS
Candida albicans	10231	10^3-10^4	N/A	Good	None
Pseudomonas aeruginosa	10145	10^3-10^4	Good	N/A	N/A
Shigella flexneri	12022	10^3-10^4	Good	N/A	N/A
Staphylococcus aureus	25923	10^3-10^4	Good	N/A	N/A
Streptococcus pneumoniae	6305	10^3-10^4	Good	Good	Alpha
Streptococcus pyogenes	19615	10^3-10^4	Good	Good	Beta
*Aspergillus brasiliensis (niger)**	16404	<100	Growth	N/A	N/A
*Bacillus subtillis**	6633	<100	Growth	N/A	N/A
*Candida albicans**	10231	<100	Growth	N/A	N/A
*Escherichia coli**	8739	<100	Growth	N/A	N/A
*Pseudomonas aeruginosa**	9027	<100	Growth	N/A	N/A
Salmonella enterica subsp. *enterica* serotype Typhimurium*	14028	<100	Growth	N/A	N/A
*Staphylococcus aureus**	6538	<100	Growth	N/A	N/A

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).*

BBL™ Trypticase™ Soy Agar (prepared bottle)

Inoculate and incubate at 35-37°C for 48 hours with 3-5% CO_2 (supplemented with sheep blood). Incubate (*) cultures at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Streptococcus pneumoniae	6305	10^4-10^5	Good
Streptococcus pyogenes	19615	10^4-10^5	Good
*Aspergillus brasiliensis (niger)**	16404	<100	Growth
*Bacillus subtillis**	6633	<100	Growth
*Candida albicans**	10231	<100	Growth
*Pseudomonas aeruginosa**	9027	<100	Growth
*Staphylococcus aureus**	6538	<100	Growth

BBL™ Trypticase™ Soy Agar (prepared plate)

Inoculate and incubate at 35 ± 2°C for 48 hours (incubate *S. pyogenes* with 3-5% CO_2). Incubate (*) cultures at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans).*

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Shigella flexneri	12022	50-100	Good
Staphylococcus aureus	25923	50-100	Good
Streptococcus pyogenes	19615	50-100	Good
*Aspergillus brasiliensis (niger)**	16404	<100	Growth
*Bacillus subtillis**	6633	<100	Growth
*Candida albicans**	10231	<100	Growth
*Pseudomonas aeruginosa**	9027	<100	Growth
*Staphylococcus aureus**	6538	<100	Growth

Inoculate *Haemophilus* strains with a 1:10 dilution from a broth culture and incubate at 35 ± 2°C with 3-5% CO_2 for 24 hours.

ORGANISM	ATCC™	TAXO X	TAXO V	TAXO XV
Haemophilus influenzae	9334	–	–	+
Haemophilus parahemolyticus	10014	–	+	+
Haemophilus parainfluenzae	9796	–	+	+

BBL™ Trypticase™ Soy Agar (prepared Sterile Pack plate)

Inoculate and incubate at 30-35°C for up to 3 days (incubate *A. brasiliensis* at 20-25°C for up to 7 days). Incubate (*) cultures at 30-35°C for up to 3 days (up to 5 days for *A. brasiliensis* and *C. albicans*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	10-100	Good
Escherichia coli	8739	10-100	Good
Kocuria rhizophila	9341	10-100	Good
Staphylococcus epidermidis	12228	10-100	Good
*Aspergillus brasiliensis (niger)**	16404	10-100	Growth
*Bacillus subtillis**	6633	10-100	Growth
*Candida albicans**	10231	10-100	Growth
*Pseudomonas aeruginosa**	9027	10-100	Growth
*Staphylococcus aureus**	6538	10-100	Growth

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.[5-7,18] Consult appropriate texts for the growth patterns produced by the various strains of *Haemophilus*.[5-7]

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. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
7. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
8. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
9. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
10. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
11. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.
12. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
13. Health Canada. The compendium of analytical methods, online. Food Directorate, Health Products and food Branch, Health Canada, Ottawa, Ontario Canada.
14. International Organization for Standardization. 1994. Microbiology – General guidance for the detection of presumptive pathogenic *Yersinia enterocolitica*. ISO 10273, 1st ed., 1994-12-15. International Organization for Standardization, Geneva, Switzerland.
15. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
16. Curry, Joyce and McEwen. 1993. CTFA microbiology guidelines. The Cosmetic, Toiletry and Fragrance Association, Inc., Washington, D.C.
17. Association for the Advancement of Medical Instrumentation. 2006. Sterilization of health care products – radiation – Part 2: Establishing the sterilization dose. ANSI/AAMI/ISO 11137-2:2006. Association for the Advancement of Medical Instrumentation, Arlington, Va.
18. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.

Availability

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

AOAC BAM CCAM COMPF EP EPA ISO JP SMD SMWW USDA USP

Cat. No.	236940	Dehydrated – 100 g†
	236950	Dehydrated – 500 g†
	236920	Dehydrated – 2 kg†
	236930	Dehydrated – 10 kg†

Europe

Cat. No.	256665	Prepared Bottles, 100 mL – Pkg. of 10
	257295	Prepared Plates – Ctn. of 100*

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

AOAC BAM CCAM COMPF EP EPA ISO JP SMD SMWW USDA USP

Cat. No.	211043	Dehydrated – 500 g†
	211046	Dehydrated – 5 lb (2.3 kg)†
	211047	Dehydrated – 25 lb (11.3 kg)†

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*

Japan

Cat. No.	251167	Prepared Plates (5 × 4) – Pkg. of 20*
	251185	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*

BBL™ Trypticase™ Soy Agar, Sterile Pack

EP JP USP

United States and Canada

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*

Europe

Cat. No.	257285	Prepared Plates (150 × 15 mm-style) – Pkg. of 5*
	257284	Prepared Plates (150 × 15 mm-style, triple bagged) – Ctn. of 30*
	254954	Prepared Plates – Pkg. of 10*
	254956	Prepared Plates – Ctn. of 100*
	257076	Prepared Plates (Deep fill) – Pkg. of 10*
	257077	Prepared Plates (Deep fill) – Ctn. of 100*

BBL™ Trypticase™ Soy Agar, Isolator Pack

United States and Canada

Cat. No.	292651	Prepared Plates – Pkg. of 10*
	292652	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	257080	Prepared Plates – Pkg. of 10*
	257081	Prepared Plates – Ctn. of 100*
	257375	Prepared Plates (Deep fill) – Ctn. of 100*
	257427	Prepared **RODAC**™ SL Plates – Ctn. of 100*
	257373	Prepared Plates (150 × 15 mm-style) – Pkg. of 5*
	257377	Prepared Plates (150 × 15 mm-style) – Ctn. of 30*
	257376	Prepared Plates – Pkg. of 10*
	257374	Prepared Plates – Ctn. of 100*

Difco™ Hycheck™ Hygiene Contact Slides

Cat. No.	290002	Tryptic Soy Agar//D/E Neutralizing Agar– Box of 10 slides*
	290003	Tryptic Soy Agar//Violet Red Bile Glucose Agar – Box of 10 slides*
	290006	Tryptic Soy Agar//Rose Bengal Chloramphenicol Agar – Box of 10 slides*
	290007	Tryptic Soy Agar with 0.01% TTC//Rose Bengal Chloramphenicol Agar – Box of 10 slides*

**Store at 2-8°C.*
†QC testing performed according to USP/EP/JP performance specifications.