

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.

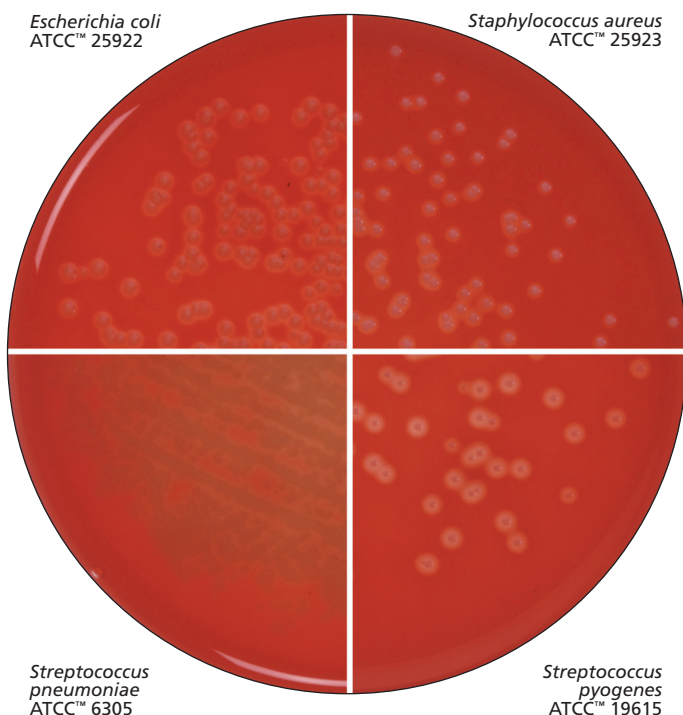
With the Sterile Pack and Isolator Pack plates, the entire double-wrapped (Sterile Pack) or triple-wrapped (Isolator Pack) product is subjected to a sterilizing dose of gamma radiation, so that the contents inside the outer package(s) are sterile.¹⁷ This allows the inner package to be aseptically removed 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% CO₂ 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 | | HEMOLYSIS |
|---|-------|-----------------|----------|------|-----------|
| | | | PLAIN | W/SB | |
| <i>Escherichia coli</i> | 25922 | 30-300 | Good | Good | Beta |
| <i>Neisseria meningitidis</i> | 13090 | 30-300 | Good | Good | None |
| <i>Staphylococcus aureus</i> | 25923 | 30-300 | Good | Good | Beta |
| <i>Streptococcus pneumoniae</i> | 6305 | 30-300 | Good | Good | Alpha |
| <i>Streptococcus pyogenes</i> | 19615 | 30-300 | Good | Good | Beta |
| <i>Aspergillus brasiliensis (niger)*</i> | 16404 | <100 | Growth | N/A | N/A |
| <i>Bacillus subtilis*</i> | 6633 | <100 | Growth | N/A | N/A |
| <i>Candida albicans*</i> | 10231 | <100 | Growth | N/A | N/A |
| <i>Escherichia coli*</i> | 8739 | <100 | Growth | N/A | N/A |
| <i>Pseudomonas aeruginosa*</i> | 9027 | <100 | Growth | N/A | N/A |
| <i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium* | 14028 | <100 | Growth | N/A | N/A |
| <i>Staphylococcus aureus*</i> | 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

- Suspend 40 g of the powder in 1 L of purified water. Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.
- For preparation of blood plates, add 5-10% sterile, defibrinated blood to the sterile agar which has been cooled to 45-50°C.
- 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% 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.

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% CO₂). 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 | | HEMOLYSIS |
|---|-------|----------------------------------|----------|------|-----------|
| | | | PLAIN | W/SB | |
| <i>Candida albicans</i> | 10231 | 10 ³ -10 ⁴ | N/A | Good | None |
| <i>Pseudomonas aeruginosa</i> | 10145 | 10 ³ -10 ⁴ | Good | N/A | N/A |
| <i>Shigella flexneri</i> | 12022 | 10 ³ -10 ⁴ | Good | N/A | N/A |
| <i>Staphylococcus aureus</i> | 25923 | 10 ³ -10 ⁴ | Good | N/A | N/A |
| <i>Streptococcus pneumoniae</i> | 6305 | 10 ³ -10 ⁴ | Good | Good | Alpha |
| <i>Streptococcus pyogenes</i> | 19615 | 10 ³ -10 ⁴ | Good | Good | Beta |
| <i>Aspergillus brasiliensis (niger)*</i> | 16404 | <100 | Growth | N/A | N/A |
| <i>Bacillus subtilis*</i> | 6633 | <100 | Growth | N/A | N/A |
| <i>Candida albicans*</i> | 10231 | <100 | Growth | N/A | N/A |
| <i>Escherichia coli*</i> | 8739 | <100 | Growth | N/A | N/A |
| <i>Pseudomonas aeruginosa*</i> | 9027 | <100 | Growth | N/A | N/A |
| <i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium* | 14028 | <100 | Growth | N/A | N/A |
| <i>Staphylococcus aureus*</i> | 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% CO₂ (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 |
|--|-------|----------------------------------|----------|
| <i>Streptococcus pneumoniae</i> | 6305 | 10 ⁴ -10 ⁵ | Good |
| <i>Streptococcus pyogenes</i> | 19615 | 10 ⁴ -10 ⁵ | Good |
| <i>Aspergillus brasiliensis (niger)*</i> | 16404 | <100 | Growth |
| <i>Bacillus subtilis*</i> | 6633 | <100 | Growth |
| <i>Candida albicans*</i> | 10231 | <100 | Growth |
| <i>Pseudomonas aeruginosa*</i> | 9027 | <100 | Growth |
| <i>Staphylococcus aureus*</i> | 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% CO₂). 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 |
|--|-------|--------------|----------|
| <i>Shigella flexneri</i> | 12022 | 50-100 | Good |
| <i>Staphylococcus aureus</i> | 25923 | 50-100 | Good |
| <i>Streptococcus pyogenes</i> | 19615 | 50-100 | Good |
| <i>Aspergillus brasiliensis (niger)*</i> | 16404 | <100 | Growth |
| <i>Bacillus subtilis*</i> | 6633 | <100 | Growth |
| <i>Candida albicans*</i> | 10231 | <100 | Growth |
| <i>Pseudomonas aeruginosa*</i> | 9027 | <100 | Growth |
| <i>Staphylococcus aureus*</i> | 6538 | <100 | Growth |

Inoculate *Haemophilus* strains with a 1:10 dilution from a broth culture and incubate at 35 ± 2°C with 3-5% CO₂ for 24 hours.

| ORGANISM | ATCC™ | TAXO X | TAXO V | TAXO XV |
|------------------------------------|-------|--------|--------|---------|
| <i>Haemophilus influenzae</i> | 9334 | – | – | + |
| <i>Haemophilus parahemolyticus</i> | 10014 | – | + | + |
| <i>Haemophilus parainfluenzae</i> | 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 |
|--|-------|--------------|----------|
| <i>Aspergillus brasiliensis (niger)</i> | 16404 | 10-100 | Good |
| <i>Escherichia coli</i> | 8739 | 10-100 | Good |
| <i>Kocuria rhizophila</i> | 9341 | 10-100 | Good |
| <i>Staphylococcus epidermidis</i> | 12228 | 10-100 | Good |
| <i>Aspergillus brasiliensis (niger)*</i> | 16404 | 10-100 | Growth |
| <i>Bacillus subtilis*</i> | 6633 | 10-100 | Growth |
| <i>Candida albicans*</i> | 10231 | 10-100 | Growth |
| <i>Pseudomonas aeruginosa*</i> | 9027 | 10-100 | Growth |
| <i>Staphylococcus aureus*</i> | 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

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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.