



# INSTRUCTIONS FOR USE – READY-TO-USE BOTTLED MEDIA

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For Laboratory Use Only

**BD Tryptic Soy Broth •**  
**BD BBL™ Trypticase™ Soy Broth•**  
**BD BBL™ Trypticase™ Soy Broth, Sterile Pack•**  
**BD Tryptic Soy Broth, Double Wrapped•**  
**BD Tryptic Soy Broth, ETO•**  
**BD Tryptic Soy Broth, Double Strength •**  
**BD Tryptic Soy Broth with 1% Polysorbate 80 •**  
**BD Tryptic Soy Broth with 0.5% Polysorbate 80, Sterile Pack**

## INTENDED USE

**Tryptic Soy Broth** and **BBL Trypticase Soy Broth** (Soybean-Casein Digest Medium) are general purpose media used in qualitative procedures for the detection, isolation, and cultivation of fastidious and nonfastidious microorganisms, in sterility testing, and in the microbiological examination of nonsterile products: microbial enumeration tests.<sup>2,3</sup> **Tryptic Soy Broth, double wrapped, Tryptic Soy Broth, ETO, and BBL™ Trypticase™ Soy Broth, Sterile Pack** are used for sterility testing in sterile filling rooms. **Tryptic Soy Broth, Double Strength**, is used for the testing of larger volumes of liquid materials. **Tryptic Soy Broth with 1% Polysorbate 80** and **Tryptic Soy Broth with 0.5% Polysorbate 80, Sterile Pack** are used for testing oils or materials containing lecithin in sterile fill rooms.

*Note that this document is valid for all catalogue numbers of the products mentioned above, intended for laboratory use.*

## PRINCIPLES AND EXPLANATION OF THE PROCEDURE

**Tryptic Soy Broth** and **BBL Trypticase Soy Broth** are nutritious media that will support the growth of a wide variety of microorganisms, including common aerobic and facultatively anaerobic bacteria and fungi.<sup>1</sup> Because of its capacity for growth promotion, this formulation was adopted by The United States Pharmacopeia (USP) and the European Pharmacopeia (EP) as a sterility test medium and for the microbiological examination of non-sterile products.<sup>2,3</sup>

In **Tryptic Soy Broth** and **BBL Trypticase Soy Broth**, enzymatic digests of casein and soybean meal provide amino acids and other complex nitrogenous substances. Glucose (=dextrose) is an energy source. Sodium chloride maintains the osmotic equilibrium. Dibasic potassium phosphate acts as a buffer to control pH.

In **Tryptic Soy Broth with 1% Polysorbate 80** and **Tryptic Soy Broth with 0.5% Polysorbate 80, Sterile Pack**, Polysorbate 80 [Polyoxyethylene (80) sorbitan monooleate] is included for testing oily materials or materials containing lecithin.<sup>2-3</sup>

## REAGENTS

### **Tryptic Soy Broth and BBL Trypticase Soy Broth**

Formula\* Per Liter Purified Water

|                               |        |
|-------------------------------|--------|
| Pancreatic Digest of Casein   | 17.0 g |
| Papaic Digest of Soybean Meal | 3.0    |
| Glucose (= Dextrose)          | 2.5    |
| Sodium Chloride               | 5.0    |
| Dipotassium Phosphate         | 2.5    |

pH 7.3 ± 0.2

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

**Tryptic Soy Broth, Double Strength**, contains all ingredients in twofold concentration. **Tryptic Soy Broth with 1% Polysorbate 80**, in addition to the ingredients listed above, contains 10 g Polysorbate 80 [Polyoxyethylene (80) sorbitan monooleate] per liter. **Tryptic Soy Broth with 0.5% Polysorbate 80, Sterile Pack**, in addition to the ingredients listed above, contains 5 g Polysorbate 80 per liter.

### **Packaging and Sterility Information**

With the exception of **Tryptic Soy Broth** provided in bags (5 or 10 liters) which are filled aseptically, the products mentioned in this document are sterilized by autoclaving in their final containers. For many of these products, a sterility claim is available on the Certificate of Analysis (<http://regdocs.bd.com> or <http://www.bd.com/europe/regulatory/>).

**Tryptic Soy Broth** in bags (5 or 10 liters) is sterilized before aseptically filling into the bags.

Each container of **Tryptic Soy Broth, double wrapped**, **Tryptic Soy Broth with 1% Polysorbate 80**, and **Tryptic Soy Broth with 0.5% Polysorbate 80, Sterile Pack** are packaged in two Stericlin® bags before autoclaving, providing a sterile outer surface of the containers.

**Tryptic Soy Broth, ETO**: after autoclaving the medium in the container, the whole package unit is sealed in a Stericlin bag and is sterilized by ethylene oxide (=ETO) treatment, providing a sterile outer surface of the containers and package.

The multiple bags in these products allow the introduction of the containers into clean rooms from a non-sterile to a sterile area without the risk of contamination.

### **PRECAUTIONS**

For laboratory use only

Do not use containers if they show evidence of microbial contamination e.g. turbidity, discoloration, drying, cracking, leakage or other signs of deterioration.

If Wide Mouth jars (closed with Twist-off lids) are opened, a popping sound must be perceived which indicates proper closure of the lid. Bottles without this popping sound upon opening must be discarded.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

### **STORAGE AND SHELF LIFE**

On receipt, store **Tryptic Soy Broth** in glass containers according to the storage temperature instructions on the label, and all **Tryptic Soy Broth in bags** at 2 to 8° C, in the dark until just prior to use. Avoid freezing and overheating. The containers may be inoculated up to the expiration date and incubated for the recommended incubation times.

### **USER QUALITY CONTROL**

Aliquot large containers (>200 ml) to smaller volumes in sterile tubes. Test samples with the organisms mentioned in the Table below. In order to achieve the USP and EP requirements, an inoculum of <100 cfu per container must be used. For details, refer to the current edition of the Pharmacopoeia.<sup>2,3</sup>

Venting of closed containers (vials, bottles, and jars) during incubation is strongly recommended to provide satisfactory growth of aerobes. Use venting needles (BD Blunt Filter needle, cat. no. 305211 or equivalent). Incubate as indicated in the Table below. Note that Tryptic Soy Broth, Double Strength, must be diluted 1:1 with sterile water before inoculation.

**Risk of secondary contamination:** Before inoculation, the outer surface of the bottles, especially the lid, cap, and/or stopper should be disinfected using a sporocidal disinfectant. Twist-off screw caps of Wide Mouth jars must be opened in a Laminar Airflow cabinet. Wear gloves that have been disinfected before opening the lid!

| Test strains                                  | Strain number   | Incubation  | Growth results |
|---|---|---|----------------|
| <i>Aspergillus brasiliensis</i> (=A. niger)** | ATCC™ 16404   | 20-25° C, <= 5 days                                   | Growth         |
| <i>Candida albicans</i>                       | ATCC 10231  | 20-25° C, <= 5 days                                   | Growth         |
| <i>Bacillus subtilis</i>                      | ATCC 6633   | 20-25° C, <= 3 days <u>and</u><br>30-35° C, <= 3 days | Growth         |
| <i>Pseudomonas aeruginosa</i>                 | ATCC 9027   | 30-35° C, <= 3 days                                   | Growth         |
| <i>Escherichia coli</i>                       | ATCC 8739   | 30-35° C, 18-24 hours                                 | Growth         |
| <i>Salmonella</i> Typhimurium                 | ATCC 14028  | 30-35° C, 18-24 hours                                 | Growth         |
| <i>Staphylococcus aureus</i>                  | ATCC 6538   | 30-35° C, <= 3 days                                   | Growth         |
| Uninoculated appearance                       | Light amber to amber, clear to very slightly opalescent. Tryptic Soy Broth, Double Strength, has an amber to brownish appearance. Media containing Polysorbate 80 may have a slightly hazy appearance. Cat. nos. 257294 and 257486 may contain inert particulate that is an inherent part of the cap liner. |   |                |

\*\**Aspergillus brasiliensis* (=A. niger) and other filamentous fungi may produce a heavy mycelium on top of the broth and mycelial fragments in the broth rather than a homogenous turbidity.

## PROCEDURE

### Materials Provided

#### BBL™ Trypticase™ Soy Broth

#### Tryptic Soy Broth,

#### Tryptic Soy Broth, double wrapped,

#### Tryptic Soy Broth, ETO,

#### Tryptic Soy Broth, Double Strength,

#### Tryptic Soy Broth with 1% Polysorbate 80,

#### Tryptic Soy Broth with 0.5% Polysorbate 80, Sterile Pack

#### Tryptic Soy Broth in bags (5, or 10 liters bag fill volume):

### Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

### Test Procedure

**Risk of secondary contamination:** Before inoculation, the outer surface of the bottles, especially the lid, cap, and/or stopper should be disinfected using a sporocidal disinfectant. Twist-off screw caps of Wide Mouth jars must be opened in a Laminar Airflow cabinet. Wear gloves that have been disinfected before opening the lid!

Tryptic Soy Broth and Tryptic Soy Broth with 1% Polysorbate 80: Inoculate the medium as soon as possible after the samples or test materials arrive at the laboratory. The way of inoculation depends on the type of test material and the type of closure of the medium bottle. For small amounts of liquid specimens, use sterile syringes for inoculating the broth medium closed with injection stoppers. Bottles with screw caps can be inoculated with solid or liquid materials.

Tryptic Soy Broth, double wrapped, Tryptic Soy Broth with 1% Polysorbate 80, double wrapped, Tryptic Soy Broth with 0.5% Polysorbate 80, Sterile Pack and Tryptic Soy Broth, ETO: These products are intended to be used in sterile filling rooms. Remove the bag(s) as they enter from the "black" (=non-sterile) into the "white" (=sterile) area. The subsequent use of the medium is identical to the one described above for Tryptic Soy Broth and Tryptic Soy Broth with 1% Polysorbate 80.

Tryptic Soy Broth, Double Strength: This medium is intended to be inoculated with liquid test materials (1:1), so that the concentration of its media ingredients is normal (see formula) after addition of the liquid material to be tested. Consult the appropriate references.

Do not use Tryptic Soy Broth, Double Strength without further dilution with the (liquid) test material!

Tryptic Soy Broth in bags is mainly used for media fill tests in validation tests. The bags provided contain at least one sterile outlet that can be connected to tubings or can be used for filling the contents into other containers.

Incubate the media as indicated above in the **USER QUALITY CONTROL** section, or as appropriate. Venting of the containers during incubation is recommended for all bottles with injection stoppers to provide satisfactory growth of aerobes. Use venting needles (BD Blunt Filter needle, cat. no. 305211 or equivalent).

For use in sterility testing, consult the USP or EP for procedural details and specifications for volume of medium relative to sample size.<sup>2,3</sup>

## Results

After incubation, growth in broth media is indicated by the presence of turbidity compared to an uninoculated control. Fungi may produce a pellicle on top of the media and specks (mycelial fragments) in the broth. Strict aerobes may also form a pellicle on top, especially in the early stage of growth. If the material tested causes turbidity of the medium, subcultures onto appropriate solid media must be performed after incubation to decide if the turbidity is caused by the material only or by micro-organisms that have multiplied in the medium.

Subcultures onto suitable solid media and biochemical and microscopic tests are necessary for the identification of the isolated organisms.

## LIMITATIONS OF THE PROCEDURE

Growth obtained in this medium must be subcultured onto appropriate solid media to obtain pure cultures which afterwards can be identified with methods appropriate for the isolate(s).

All media mentioned in this document are not the appropriate media for the detection and recovery of strict anaerobes. Instead, Fluid Thioglycollate Media should be used for this purpose.

Use of these media for clinical specimens has not been validated.

## REFERENCES

1. Marshall, R.T. (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. U.S. Pharmacopeial Convention, Inc. The U.S. Pharmacopeia /The national formulary *Current edition*. U.S. Pharmacopeial Convention, Inc., Rockville, Md
3. Council of Europe. European Pharmacopoeia, *current edition*. European Pharmacopoeia Secretariat. Strasbourg/France.

## PACKAGING/AVAILABILITY

For container types, fill volumes, package sizes, and for availability of these products, please contact your local BD representative.

## FURTHER INFORMATION

For further information please contact your local BD representative.



**Becton Dickinson GmbH**

Tullastrasse 8–12

69126 Heidelberg/Germany

Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16

Reception\_Germany@bd.com

<http://www.bd.com>

<http://www.bd.com/europe/regulatory/>

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