



INSTRUCTIONS FOR USE – READY-TO-USE PLATED MEDIA

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For Laboratory Use Only

BD BBL™ IC-XT Pack Trypticase™ Soy Agar, 90 mm LL •
BD BBL™ IC-XT Pack Trypticase™ Soy Agar with Lecithin and
Polysorbate 80, 90 mm LL •
BD BBL™ IC-XT Pack Trypticase™ Soy Agar with Lecithin and
Polysorbate 80, 150 mm •
BD BBL™ IC-XT Pack Trypticase™ Soy Agar with Lecithin and
Polysorbate 80, RODAC™ Locking Lid (LL)

INTENDED USE

Trypticase™ Soy Agar (TSA, Soybean-Casein Digest Agar Medium) is a general purpose medium which supports the growth of non-fastidious as well as moderately fastidious microorganisms. TSA is not the medium of choice for fastidious anaerobes. **Trypticase™ Soy Agar with Lecithin and Polysorbate 80** is used for the detection of microorganisms surviving after treatment of surfaces and materials with antiseptics. “IC-XT Pack” (Isolator Cleanroom-Extended Temperature) products are available with either TSA or TSA with Lecithin and Polysorbate 80 in different plate formats; they are gamma-sterilized after the aseptic fill procedure to allow monitoring of the environmental and product hygiene and the efficiency of disinfection in clean rooms of pharmaceutical production and fill rooms, and in isolators.

IC-XT products are used for monitoring the hygiene on surfaces or the microbiological quality of the air in controlled environments such as fill rooms or in isolators. All IC-XT products are packaged in impermeable plastic films to allow an extended stability and storage at 2 to 25° C throughout the shelf life. RODAC™ (Replicate Organism Detection and Counting) plates are particularly recommended for the use in the detection and enumeration of microorganisms present on surfaces of sanitary importance.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

The nutritional composition of **Trypticase™ Soy Agar** has made it a popular medium for many years. It is the medium specified as Soybean-Casein Digest Agar Medium in the United States Pharmacopeia and in the European Pharmacopeia for the total aerobic microbial count portion of the microbial limit testing procedures.^{1, 2} It is included in the compendia of methods for the examination of water, wastewater and foods.^{3, 4} TSA contains peptones which provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Sodium chloride provides osmotic equilibrium.

TSA with Lecithin and Polysorbate 80, in addition to the ingredients included in TSA, contains lecithin and polysorbate 80 to neutralize surface disinfectants.⁵⁻⁸ Lecithin is a neutralizer of quaternary ammonium compounds. Polysorbate 80 neutralizes phenols, hexachlorophene, formalin and, with lecithin, ethanol.⁹⁻¹¹

Sodium pyruvate is added to both formulations to absorb peroxides and radicals that develop during gamma-irradiation and during exposure to isolator air that contains residues of hydrogen peroxide.

The aseptic manufacturing processes of these media are controlled to ensure that the bioburden of the product is reduced to a minimum. Each piece of equipment used in the manufacturing has been qualified and validated. Using a proprietary filling process, Isolator Pack media are dispensed in a controlled environment, which has been verified as ISO class 5 and is monitored during production to assure that specifications are met. Once a medium is dispensed, the plates of all IC-XT products are packed and sealed in a dedicated, controlled environment (ISO class 7)

into three impermeable plastics bags to reduce evaporation and oxidation of the medium to a minimum. This allows storage at room temperature for the whole shelf life period.

Because the entire triple-bagged product in its carton box is subjected to a sterilizing dose of gamma-irradiation, the contents inside the outer bag are sterile. This allows the inner bags to be aseptically removed and brought into an environmental-controlled area without introducing contaminants.

The microbiological status of these products has been validated according to ISO 11137.^{12,13} As a result from the validation tests, an irradiation dose of 9.6 kGy was determined to be the minimum irradiation dose necessary for achieving an SAL of 10^{-5} .¹⁴ The media are gamma-irradiated in the packaging material as delivered with 10 to 22 kGy to assure a reduction of the microbial load potentially present in the medium, on the dishes, and on the packaging materials. Gamma-irradiation of the product is indicated by an orange to red color of the irradiation indicator stripe on the inner label. A yellow to mustard-colored indicator indicates insufficient irradiation.

The bags (with undamaged sealing seams) of the IC-XT products are impermeable to hydrogen peroxide. This applies to product packaged in one, two or three bags.

REAGENTS

Approximate Formulas* Per Liter Purified Water

All IC-XT products with **BD BBL™ Trypticase™ Soy Agar**

Pancreatic Digest of Casein	15.0 g
Papaic Digest of Soybean Meal	5.0
Sodium Chloride	5.0
Sodium Pyruvate	3.5
Agar	15.0

pH 7.3 ± 0.2

All IC-XT products with **BD BBL™ Trypticase Soy Agar with Lecithin and Polysorbate 80**

Pancreatic Digest of Casein	15.0 g
Papaic Digest of Soybean Meal	5.0
Lecithin	0.7
Polysorbate 80	5.0
Sodium Chloride	5.0
Sodium Pyruvate	3.5
Agar	17.0

pH 7.3 ± 0.2

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

PRECAUTIONS

For laboratory use only.

The contents of the unopened and undamaged bags are sterile. Do not use packages if they show evidence of microbial contamination, discoloration, drying, cracking, open or damaged bags or other signs of deterioration. The inner bag of these products contains irradiation indicator dots or stripes (dark orange to red = irradiated; yellow to mustard-colored = not irradiated). Do not use the product if the irradiation indicators are yellow to mustard-colored!

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

Packaging Information

10 plates each of these products are packed in three plastic bags. The plastic bags used for packaging of these products consist of polyethylene/ polyethylene terephthalate (=PE/PET). The inner plastic bag contains a SORB-IT® silica gel desiccant bag. White cartons are used for packaging the bagged stacks.

The sealing seams of the bags are heat-sealed. The bags allow easy opening without the use of sharp objects such as scissors or knives. Bags can be peeled open at the ends of the stacks by

tearing apart both plastic films of the bag. Apply aseptic techniques. Once the outer bag is opened, appropriate measures should be used to maintain the sterility of the inner bags and the contents.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 25° C, in their original bags until just prior to use. Do not freeze or overheat! Avoid repeated and/or extreme variations in temperature during storage since this may cause the development of excessive moisture in the bags and plates. The ideal storage temperature of these products is 15 to 22° C. Moisture appearing as a fine haze or as small droplets on the inner side of the lids, especially during or after refrigerated storage, is acceptable and is a sign for freshness of the media. Minimize exposure to light during the whole storage period.

The plates may be inoculated up to the expiration date and incubated for the recommended incubation times. The given shelf life and expiry applies to the product in unopened (completely sealed) bags.

USER QUALITY CONTROL

Inoculate representative samples of the medium with <100 CFU per plate of the strains listed in the Table. Use **Trypticase Soy Agar** as a growth reference medium. See Table for incubation. After the incubation, compare the CFU on both media (see Table footnote). The recovery on the test medium must be > 70% as compared to the reference medium.

Species	Strains	Incubation	Expected Recovery (%)*
<i>Aspergillus brasiliensis</i>	ATCC™ 16404	2-5 d/20-25° C 2-5 d/30-35° C	>70
<i>Candida albicans</i>	ATCC 10231	2-5 d/20-25° C 2-5 d/30-35° C	>70
<i>Bacillus subtilis</i>	ATCC 6633	1-3 d/30-35° C	>70
<i>Escherichia coli</i>	ATCC 8739	1-3 d/30-35° C	>70
<i>Pseudomonas aeruginosa</i>	ATCC 9027	1-3 d/30-35° C	>70
<i>Salmonella Typhimurium</i>	ATCC 14028	1-3 d/30-35° C	>70
<i>Staphylococcus aureus</i>	ATCC 6538	1-3 d/30-35° C	>70
<i>Staphylococcus epidermidis</i>	ATCC 12228	1-3 d/30-35° C	>70
Appearance of the uninoculated medium	Colorless to light amber, clear to slightly hazy		

* Recovery (%) = $\text{CFU}_{\text{Test medium}} / \text{CFU}_{\text{Reference medium}} \times 100$

PROCEDURE

Materials Provided

See **PACKAGING/AVAILABILITY** for the available IC-XT products.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Test Procedure

IC-XT products are used in a variety of procedures. Follow the appropriate references for sampling, inoculation, and incubation.¹⁻⁴

Locking Lid (LL) plates feature a locking system specifically designed to ensure higher security and convenience throughout handling while reducing the risk of accidental contamination. The LL mechanism allows for an easy locking of plates after sampling and for a safer transport from a controlled environment to the laboratory. The locked position of the plate ensures a secure fit between the lid and the base thereby minimizing unintentional opening of plates while allowing for appropriate aeration during incubation.

The **RODAC™ Locking Lid (LL) plated media** are used in the replicate organism detection and counting procedure to monitor the hygiene status of surfaces or in certain types of air samplers.

For surface testing, introduce the plates into the room or area to be tested or monitored. RODAC™ LL plates are provided in the unlocked position. Remove lid from the plate. Apply the plate's surface directly to the surface being tested and exert moderate pressure. Do not rub the agar surface or move laterally on the test surface! Return the lid and lock the plate by simply twisting the base and the lid of the plate into the locked position. Plates can be easily unlocked by untwisting. Areas (walls, floors etc.) to be tested may be divided into sections or grids and samples taken from specific points within the grid.

Grid method:

1. Subdivide surface (floor or wall) into 36 equal squares per 100 square feet of area by striking five equidistant dividing lines from each of two adjacent sides.
2. These dividing lines intersect at twenty-five points.
3. Number these intersections consecutively in a serpentine configuration.
4. Use red numerals for odd numbers, black numerals for even numbers.
5. Omit number 13 which falls in the center of the total area.
6. Sample odd points at one sampling period, even points at the next sampling period.
7. For areas greater than 100 square feet, extend grid to include entire area.
8. 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.
9. For areas between 25 and 100 square feet, divide into 36 equal squares as in #1.
10. Mark plates with intersection numbers.

Products supplied in 90 mm LL dishes are used in general microbiological procedures or for monitoring the finger hygiene ("finger dabbing"). The 90 mm LL plates contain 25 grams of medium and are used in laminar air flow cabinets. The large amount of medium reduces the evaporation and shrinkage caused by the air flow venting (see **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE** for further information).
Products supplied in 150 mm dishes are generally used in air sedimentation procedures.

Incubate inoculated plates at 30 to 35 ° C and at 20 to 25° for up to 5 days or as required.

Results

After the incubation, viable microorganisms will produce colonies on the surface of the medium that should be counted. Counting of plates containing a profusion of growth can lead to considerable error. A basic decision to be made is whether distinct colony margins can be observed. 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 or halo-type spreaders.

From the isolates obtained on the media, appropriate subcultures should be set up to allow a further differentiation and identification. Refer to appropriate references and procedures.¹⁻³

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

These media are intended for the enumeration of microorganisms in hygiene control and on surfaces of sanitary importance.

Performance Results

External Performance Evaluation

In two external performance studies, the neutralization efficiency of **Trypticase™ Soy Agar with Lecithin and Polysorbate 80** was evaluated. In these studies, 13 microorganisms and 24 disinfectants with different active ingredients were tested. These studies indicated that the product neutralizes the tested disinfectants and allows satisfactory growth of the tested microorganisms in the presence of disinfectants¹⁸.

Limitations of the procedure

Trypticase™ Soy Agar and TSA with Lecithin and Polysorbate 80 are not suitable media for fastidious bacteria and are not the media of choice for fastidious anaerobes.

Trypticase™ Soy Agar does not contain compounds that actively neutralize disinfectants or preservatives. If materials containing such compounds or surfaces that have been previously disinfected shall be monitored, it is recommended to use media containing **TSA with Lecithin and Polysorbate**.

Extended sedimentation exposure followed by incubation in dry air may lead to cracking or splitting of the agar gel.

These media do not allow a complete identification. Further tests, made from pure cultures of the isolates, must be performed for complete identification of the isolated microorganisms. Consult the references.¹⁵⁻¹⁷

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

REFERENCES

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18. Data on file: Neutralization efficiency studies

PACKAGING/AVAILABILITY

Product Name	Cat. No.	Number of plates per package
BD BBL™ IC-XT Pack Trypticase™ Soy Agar, 90 mm LL	257632	100
BD BBL™ IC-XT Pack Trypticase™ Soy Agar with Lecithin and Polysorbate 80, 90 mm LL	257635	100
BD BBL™ IC-XT Pack Trypticase™ Soy Agar with Lecithin and Polysorbate 80, 150 mm	257636	30
BD BBL™ IC-XT Pack Trypticase™ Soy Agar with Lecithin and Polysorbate 80, RODAC™ Locking Lid (LL)	257637	100

FURTHER INFORMATION

For further information please contact your local BD representative.



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