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

INTENDED USE

Trypticase™ Soy Agar with Lecithin and Polysorbate 80 with Penase is used for the detection of micro-organisms surviving after treatment of surfaces and materials with antiseptics in penicillin filling rooms.

"**IC-XT Pack**" (Isolator Cleanroom-Extended Temperature) products are available 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. 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 micro-organisms present on surfaces of sanitary importance.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

The nutritional composition of Trypticase™ Soy Agar used in **BD BBL™ IC-XT Pack Trypticase™ Soy Agar with Lecithin and Polysorbate 80 with Penase** 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. Lecithin and Polysorbate 80 are specifically included 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 absorb peroxides and radicals that develop during gamma-irradiation and during exposure to isolator air that contains residues of hydrogen peroxide.

BD BBL™ IC-XT Pack Trypticase™ Soy Agar with Lecithin and Polysorbate 80 with Penase contains Penicillinase Concentrate to inactivate penicillin dust that may accumulate on the surface of media used for air sampling and in sedimentation procedures. The inactivation spectrum of the penicillinase when added to this medium includes penicillin G, mezlocillin, oxacillin, and first generation cephalosporins such as cefazolin when tested by the agar diffusion method using *Staphylococcus aureus* ATCC 29737 as an indicator strain and **Trypticase Soy Agar** as a reference medium.

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 process 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 with Lecithin and Polysorbate 80 with Penase**

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
Penase	50 ml

pH 7.3 ± 0.3
*Adjusted and/or supplemented as required to meet performance criteria.

BD BBL™ Trypticase Soy Agar with Lecithin and Polysorbate 80 with Penase contains 50 ml of **Penicillinase Concentrate** per liter medium, added aseptically before gamma irradiation. Penicillinase Concentrate has a potency of 10 million Kershey units/ml. According to the kinetic method of Kershey et al., 1 unit of benzyl penicillinase will inactivate 0.39 units of benzyl penicillin per hour in phosphate buffer at pH 7.0, at 30° C.[15] The penicillinase in Penicillinase Concentrate is obtained from a specific strain of *Bacillus cereus*.

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. The triple-bagged stacks are packaged in white cartons.
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 (colony forming units) per plate of the strains listed in the Table. Use **BD 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 (%)***
Aspergillus brasiliensis	ATCC 16404	2-5 d/30-35° C	>70
Candida albicans	ATCC 10231	2-5 d/30-35° C	>70
Bacillus subtilis	ATCC 6633	1-3 d/30-35° C	>70
Escherichia coli	ATCC 8739	1-3 d/30-35° C	>70
Pseudomonas aeruginosa	ATCC 9027	1-3 d/30-35° C	>70
Salmonella Typhimurium	ATCC 14028	1-3 d/30-35° C	>70
Staphylococcus aureus	ATCC 6538	1-3 d/30-35° C	>70
Staphylococcus epidermidis	ATCC 12228	1-3 d/30-35° C	>70
Appearance of the uninoculated medium	Light to medium tan yellow and clear to trace hazy		

* Recovery (%) = $CFU_{\text{Test medium}}$ / $CFU_{\text{Reference medium}}$ x 100

Additionally, the penicillinase activity in **BD BBL™ Trypticase Soy Agar with Lecithin and Polysorbate 80 with Penase** is tested as follows: Prepare a suspension matching the McFarland standard 0.5 (approximately 5 x 10^7 to 10^8 CFU/ml) of *S. aureus* ATCC 29737 from an overnight culture on Trypticase Soy Agar. Swab-inoculate the whole surface of the test medium with this suspension and place appropriate sensitivity test discs (e.g. **BD BBL Sensi-Discs**) on the surface of **BD BBL™ Trypticase Soy Agar with Lecithin and Polysorbate 80 with Penase**. **BD Trypticase Soy Agar** plates may be prepared in the same way as a reference medium. Incubate aerobically for 18 to 24 hours at 35 to 37° C and measure the zone diameter. Zones on the medium with and without penicillinase are shown in the Table below:

Test strain	**Antimicrobial**	**BD BBL™ Trypticase Soy Agar with Lecithin and Polysorbate 80 with Penase**	**BD Trypticase Soy Agar**
Staphylococcus aureus ATCC 29737	Penicillin P-10	No zone	Clear zone >/= 35 mm
	Cefazolin CZ-30	Turbid zone < 13 mm	Clear zone >/= 30 mm

For the evaluation of disinfectant and preservative neutralization, spread-inoculate **BD BBL™ Trypticase Soy Agar with Lecithin and Polysorbate 80 with Penase** with the test strains (starting from suspensions matching the McFarland 0.5 standard) and place filter paper discs containing working concentrations of the disinfectants or antiseptics in use onto the inoculated

plates. Include **BD Trypticase Soy Agar** as a reference medium. Incubate and determine the inhibition zones on the test and on the reference medium. **BD BBL™ Trypticase Soy Agar with Lecithin and Polysorbate 80 with Penase** should exhibit no zones or zones markedly smaller than the reference medium if the media are effective in neutralizing the respective disinfectants.

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 for monitoring the hygiene in penicillin filling rooms by the air sampling or sedimentation methods. 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. Place plates with lids removed in the area under test. Exposure time must be validated internally. Avoid excessive desiccation of the media which may be enhanced by ventilation!

Incubate plates at 30 to 35° C 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]

LIMITATIONS OF THE PROCEDURE

These media are intended for the enumeration of organisms in hygiene control and on surfaces of sanitary importance. **Trypticase™ Soy Agar and TSA with Lecithin and Polysorbate 80 with Penase** are not suitable media for fastidious bacteria and are not the media of choice for fastidious anaerobes.
Extended sedimentation exposure followed by incubation in dry air may lead to cracking, splitting or other desiccation of the agar gel, especially in dry environments. Media shrinkage may also occur during extended incubation in incubators with air circulation. Provide sufficient moisture during incubation since media shrinkage may affect the fertility of the medium.
If new disinfectants are used, the media must first be validated for neutralization.
The penicillinase in **Trypticase™ Soy Agar and TSA with Lecithin and Polysorbate 80 with Penase** inactivates first generation penicillins, mezlocillin, oxacillin, and first generation cephalosporins. Newer cephalosporins, carbapenems, monobactams, and combinations of beta lactams with beta lactamase inhibitors are not necessarily inactivated. If such antimicrobials shall be inactivated, their suitability must first be validated by the user.
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 micro-organisms. Consult the references.[16-18]
Use of this medium with clinical specimens has not been validated.

REFERENCES

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PACKAGING/AVAILABILITY

Product Name	Cat. No.	Number of plates per package
BD BBL™ IC-XT Pack Trypticase™ Soy Agar with Lecithin and Polysorbate 80 with Penase, 90 mm LL	257633	100
BD BBL™ IC-XT Pack Trypticase™ Soy Agar with Lecithin and Polysorbate 80 with Penase, RODAC™ Locking Lid (LL)	257634	100

FURTHER INFORMATION

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