IFU

Instructions for Use

TRYPTIC SOY AGAR (TSA), USP, IRRADIATED

Cat. no. P365	Tryptic Soy Agar (TSA), with Lecithin and Tween® 80, USP, Irradiated, Triple Bagged, Contact Plate, Lok-Tight™, 15ml	10 plates/bag
Cat. no. P365R	Tryptic Soy Agar (TSA), with Lecithin and Tween® 80, USP, Irradiated, Triple Bagged, Red-Tinted Contact Plate, Lok-Tight™, 15ml	10 plates/bag
Cat. no. W365	Tryptic Soy Agar (TSA) with Lecithin and Tween® 80, USP, SterEM™, Irradiated, Triple Bagged, 15x100mm Plate, 26ml	10 plates/bag
Cat. no. W340	Tryptic Soy Agar (TSA), USP, SterEM™, Irradiated, Triple Bagged, 15x100mm Plate, 26ml	10 plates/bag
Cat. no. W340R	Tryptic Soy Agar (TSA), USP, SterEM™, Irradiated, Triple Bagged, Red-Tinted 15x100mm Plate, 26ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Tryptic Soy Agar (TSA), USP, Irradiated is recommended for use as a general growth medium for the detection and enumeration of microorganisms in the microbiological examination of non-sterile products. Tryptic Soy Agar (TSA) with Lecithin and Tween®, USP, Irradiated is recommended for use as a general growth medium for establishing microbiological trends, alerts, and action levels in biologically controlled environments.

The media are formulated according to the United States Pharmacopoeia (USP) <62> and meet the harmonized USP/EP/JP standards for the microbial examination of non-sterile products.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

The formulation of Tryptic Soy Agar, USP is prepared according to the *United States Pharmacopoeia* standard formula for Soybean-Casein Digest Agar Medium.[(2)]

Hardy Diagnostics Tryptic Soy Agar (TSA) contains digests of soybean meal and casein which provide amino acids and other nitrogenous compounds to promote the growth of a variety of microorganisms. Sodium chloride is added to maintain the osmotic equilibrium. Agar is the solidifying agent. Lecithin and Tween® 80 are added to neutralize the antimicrobial effects of disinfectants or cleaning solutions used on environmental surfaces.

The plates are triple bagged and sterilized by irradiation to promote a higher sterility assurance level.

FORMULA

Ingredients per liter of deionized water:*

TSA contains:	

Pancreatic Digest of Casein	15.0gm
Peptic Digest of Soybean Meal	5.0gm
Sodium Chloride	5.0gm
Agar	15.0gm
TSA with Lecithin & Tween® 80 also contains:	
Lecithin	0.7gm
Tween® (polysorbate) 80	5.0ml

Final pH 7.3 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Store at 15-30°C., and store all media away from direct light. Media should not be used if there are any signs of contamination, deterioration, discoloration, or if the expiration date has passed. Product is light and temperature sensitive. Protect from freezing.

Small amounts of condensation may be observed upon receipt, especially during seasonal (summer, winter, heat waves, etc.) weather shipments. If present, condensation should improve over time when the media is stored long-term under the labeled storage conditions.

Inspect the packaging seals prior to opening and using the product. Do not use irradiated media if there is any damage to the packaging or breach of seals prior to use.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Before Use: Due to the triple packaging, it is possible variations in temperature and pressure during shipping and storage may cause condensation within the innermost sleeve surrounding the plates. If condensation of the packaging or plates is observed just prior to use, remove the plates from the innermost packaging in a controlled environment (e.g. ISO 5) and allow them to dry for 10-15 minutes before use.

The medium should be room temperature and the surface dry prior to use. To reduce the potential for cross-contamination, it is strongly suggested that appropriate gowning and glove procedures, designated aseptic processing areas, appropriate sporicidal disinfectants, and environmental monitoring procedures be strictly followed to reduce the likelihood of accidental contamination.(1)

Method of Use: For environmental monitoring procedures, consult USP <1116> *Microbiological Evaluation of Clean Rooms and Other Controlled Environments.*(1) Incubate media using appropriate atmospheric, temperature, and duration conditions as outlined by the test method.(1) Count the number of colonies and report as the number of colony forming units (CFU).

Sedimentation Plate Method: Place the plate on a clean surface and expose the agar by removing the lid. Do not invert the lid while removed to avoid exposure to falling sediment. Expose the agar for 15 minutes or longer, depending upon established procedures, and replace the lid. Incubate according to laboratory protocol.

Impact Air Sampling Method: Use the plate size specified for the impact air sampling unit. Remove the sampler head and place the plate, lid up, into the slot. Aseptically remove the lid and expose the agar; do not invert the lid while removed to avoid exposure to falling sediment. Place the sampler head back on the unit and turn the unit on; sample a specific volume of air according to laboratory procedure. After sampling, remove the sampler head, aseptically return the lid of the plate and remove the plate from the sampling unit; incubate per laboratory protocol.

Contact Plate Method of Use: Select a surface to test. Sample the surface by firmly pressing the agar against the test area, using the thumb and second finger to hold the plate, and the first finger to press firmly and evenly on the base. The same amount of pressure should be used for each sample. Do not move the plate laterally, as this spreads contaminants across the agar surface. A rolling motion may be used when slightly curved surfaces are sampled. Areas to be assayed may be divided into grids or sections, and samples taken from specific areas within the divisions. Incubate per laboratory protocol.

Personnel Sampling Method of Use: Gloved finger tips can be sampled using 15x100mm plates. Gently press each finger of the gloved hand onto the surface of the agar, being careful not to puncture or break the agar surface. Gowns and sleeves can be sampled using contact plates. Both samples can be quantified as CFU per five fingers per hand or as CFU per 25cm^2.

INTERPRETATION OF RESULTS

Consult the appropriate reference for information regarding further testing and identification of microbial cultures.(1-2)

Because of the inherent variability of environmental sampling methods, it is more useful to trend contamination recovery results rather than focus on the number of colonies recovered from a single sample. Action should be required when the contamination recovery rate trends above the recommended action levels for a significant time.

If action levels have been identified, a thorough investigation into the adequacy of personnel work practices, operational procedures, cleaning procedures and solutions, and air filtration efficiency within the processing area must be made. Once changes have been made, monitoring procedures must be repeated to determine if the changes made were effective. Documentation of all monitoring results, remedial action and follow-up monitoring must be maintained. Consult listed reference for more detailed information concerning plate count methods.(1)

Counting Colonies: After incubation, colonies are counted and results expressed as the number of CFU. For contact plates, the media surface area is approximately 25cm^2. The number of colonies can be expressed as the number of CFU per plate or divided by 25 to provide the estimated number of colonies per square centimeter.(1,2) Similarly, for 15x100mm plates, the media surface area is approximately 55cm^2. The number of counted colonies can be expressed as the number of CFU per plate, per unit of time, or per volume of air sampled based on laboratory sampling method.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Avoid repeated and/or extreme variations in temperatures during storage, as this can cause the release of excessive moisture from the media in the bags and plates.

Accurate counting may be difficult with molds or spreading colonies.

Sampling challenges may occur with irregular, porous, rough or textured media surfaces.

Contact plate media are not recommended for sampling crevices or irregular surfaces.

If removing the outer and middle packaging and storing plates in the innermost sleeve long-term in a controlled environment, 2-8°C storage conditions are recommended to prevent dehydration of the medium prior to use.

Storage at 2-8°C in the triple packaging may result in moisture build-up inside sealed packs.

Each sleeve of media includes a label with an irradiation indicator strip that turns from yellow to orange upon irradiation exposure (either the entire strip or just a portion). Inspect strips for evidence of a color change, which indicates successful irradiation. **Note**: the color intensity of indicator strips may fade or change if the label is exposed to ambient light during long-term storage. Protect from light and examine for color change immediately upon exposure to ambient lighting.

The product packaging has been verified to be resistant to hydrogen peroxide (H_2O_2) decontamination cycles at a concentration of 35% for 30 minutes. A single cycle decontamination simulation was performed using VHP (vaporized H_2O_2) and NHP (aerosolized/nebulized H_2O_2) on triple wrapped and double wrapped (outer wrap removed) samples. Growth promotion was performed on the H_2O_2 treated samples one hour after decontamination exposure using USP quality control strains and dilutions and compared to an untreated control. All H_2O_2 treated samples and untreated controls met USP <61> growth recovery criteria. However, due to variability in H_2O_2 decontamination methods, it is recommended users consider performing an internal validation.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, impact air samplers, sterile sample bags (Cat. no. BAS381BX), applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms**	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Staphylococcus aureus ATCC® 6538	J	1-3 days	30-35°C	Aerobic	Growth
Candida albicans ®	J	1-5 days	30-35°C	Aerobic	Growth

ATCC 10231					
Bacillus spizizenii ATCC® 6633	J	1-3 days	30-35°C	Aerobic	Growth
Aspergillus brasiliensis ATCC® 16404	J	1-5 days	30-35°C	Aerobic	Growth
Pseudomonas paraeruginosa ATCC® 9027	J	1-3 days	30-35°C	Aerobic	Growth
Also included for Cat. nos. P365 and P365R:					
Staphylococcus epidermidis ATCC® 12228	A	24 hr	30-35°C	Aerobic	Growth
Clostridium sporogenes ATCC® 19404	A	24-48 hr	30-35°C	Anaerobic	Growth

Representative samples from each lot of irradiated media are held for seven days to confirm the media meet the validated sterilization process sterility assurance level (SAL) of 10^{-6} following ANSI/AAMI/ISO 11137.

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Tested in accordance with USP <61> and <62>.(2)

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Tryptic Soy Agar (TSA) Agar should appear translucent, and light amber in color.

Tryptic Soy Agar (TSA) Agar with Lecithin and Tween® 80 should appear clear to slightly hazy and light amber in color.



Uninoculated plate of Tryptic Soy Agar, Irradiated.

REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, APHA, Washington, D.C.

2. The Official Compendia of Standards. *USP-NF*. United States Pharmacopeial Convention, Rockville, MD.

3. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

ATCC is a registered trademark of the American Type Culture Collection.
Tween is a registered trademark of ICI Americas, Inc.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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