IFU

Instructions for Use

TRYPTIC SOY AGAR (TSA) WITH CYCLOHEXIMIDE

Cat. no. G70	TSA with Cycloheximide, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Tryptic Soy Agar with Cycloheximide is recommended for use as a general growth medium for the isolation and cultivation of microorganisms while inhibiting Saprophytic fungal organisms.

SUMMARY

The formulation of Tryptic Soy Agar is prepared according to the United States Pharmacopeia (USP) standard formula for Soybean-Casein Digest Agar Medium.[9] Tryptic Soy Agar contains digests of soybean meal and casein, making it suitable for the growth of a wide variety of fastidious and nonfastidious microorganisms. The combination of soy and casein peptones supply organic nitrogen in the form of amino acids and polypeptides, making the medium highly nutritious. Sodium chloride is added to maintain the osmotic equilibrium. Agar is the solidifying agent. The addition of cycloheximide makes it a selective medium inhibiting Saprophytic fungal organisms that may be present in a mixed flora sample.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	15.0gm
Peptic Digest of Soybean Meal	5.0gm
Sodium Chloride	5.0gm
Cycloheximide	50.0mg
Agar	15.0gm

Final pH 7.3 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

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

Specimen Collection: Consult listed references for information on specimen collection.[1,2,5] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium and refrigerated until inoculation.

Method of Use: Plates should be warmed to room temperature and the agar surface should be dry prior to inoculating. Inoculate and streak the specimen as soon as possible after collection.

If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop. Incubate plates aerobically at 35-37ºC. for 18-24 hours (some organisms may take longer than 24 hours for visible growth to appear). Examine for colonial morphology.

Spread Plate Method:

1. Prepare decimal dilutions in sterile diluent to obtain 30-300 CFU per plate.

2. Aseptically inoculate agar surface with 0.1ml of well-mixed diluted sample.

3. Using a sterile spreader device, distribute the inoculum evenly over the agar surface.

4. Incubate plates aerobically for 1 to 5 days at 35ºC.

INTERPRETATION OF RESULTS

Consult listed references for the identification of colony morphology and further biochemical tests required for identification.[1-5]

Following incubation, examine the plates for growth. Count the number of colonies and express in number of colony forming units (CFU) per gram or milliliter of sample. (Take into account the dilution factor.) If duplicate plates were set up, express the average for the two plates in terms of the number of microorganisms per gram or milliliter of sample. Consult listed references for additional information on interpretation and enumeration of microbial growth on

this medium.[2-5,8]

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.

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, swabs, applicator sticks, other culture media, 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® 25923	A	18-24hr	35°C	Aerobic	Growth
Escherichia coli ATCC® 25922	A	18-24hr	35°C	Aerobic	Growth
Candida albicans ATCC® 10231	A	18-24hr	35°C	Aerobic	Growth
Aspergillus brasiliensis ATCC® 16404	G	48hr	20-25°C	Aerobic	Partial to complete inhibition

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

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.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

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

5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

6. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*, Lipincott Williams & Wilkins, Philadelphia, PA.

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

8. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.

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

IFU-10810[C]

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

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