TRYPTIC SOY AGAR (TSA) WITH LECITHIN AND TWEEN® 80

Cat. no. P45	TSA with Lecithin and Tween® 80, 15x60mm Contact Plate, 15ml	20 individually bagged plates/box
Cat. no. Q13	TSA with Lecithin and Tween® 80, 20x125mm Tube, 18ml Deep	20 tubes/box
Cat. no. U174	TSA with Lecithin and Tween® 80, 500ml Polycarbonate Bottle, 400ml	10 bottles/box
Cat. no. U412	TSA with Lecithin and Tween® 80, 16oz. Glass Bottle, 400ml	12 bottles/box
Cat. no. W41	TSA with Lecithin and Tween® 80, 15x100mm Plate, 27ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Tryptic Soy Agar (TSA) with Lecithin and Tween® 80 is recommended for the isolation of microorganisms from environmental surfaces and is used primarily to monitor microbial contamination, enumerate the number of microbial colonies growing on a variety of surfaces sanitized with quaternary ammonium compounds, and to assist in determining surface sanitation.

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

SUMMARY

Hardy Diagnostics Tryptic Soy Agar (TSA) with Lecithin and Tween® 80 contains digests of soybean meal and casein that provide amino acids and other nitrogenous compounds, making it a nutritious medium for many microorganisms. Sodium chloride is added to maintain cellular osmotic equilibrium. Lecithin and Tween® 80 are added to the formulation to neutralize germicidal or disinfectant residues. Neutralization of these residues reduces their inhibitory

effect which ultimately results in lowering of microbial count. Quaternary ammonia compounds are neutralized by lecithin, while phenolic disinfectants and hexachlorophene are neutralized by Tween® 80. Together, lecithin and Tween® 80 neutralize ethanol.[1,2]

When the contact surface is flat and the expected number of recoverable microbes is between 30-300 colonies, the contact plate method is most useful. The contact plate method was one of several methods developed in the 1930's to monitor surfaces for contamination. In this method, a petri dish with a specified grid molded into the bottom of the plate and a diameter of 60mm is slightly overfilled with nutritive media. Surface contact is made easy by raising the meniscus above the rim.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	15.0gm
Peptic Digest of Soybean Meal	5.0gm
Sodium Chloride	5.0gm
Tween® 80	5.0gm
Lecithin	0.7gm
Agar	15.0gm

Final pH 7.3 +/- 0.2 at 25 degrees C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8 degrees C., store Cat. no. P45 at 15-30 degrees C., 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 applies to the product in its intact packaging when stored as directed.

This product has the following shelf life from the date of manufacture:

82 Days:	P45	TSA with Lecithin and Tween® 80
90 Days:	W41	TSA with Lecithin and Tween® 80
180 Days:	U174	TSA with Lecithin and Tween® 80
	U412	TSA with Lecithin and Tween® 80
365 Days:	Q13	TSA with Lecithin and Tween® 80

Refer to the keyword "Storage", in the Hardy Diagnostics' software program HUGO™, for more information on storing culture media.

PRECAUTIONS

This product is for laboratory use only and 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". The "Guideline for Isolation Precautions" is available from the Centers of Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the keyword "Precautions", in the Hardy Diagnostics' software program HUGO™, for more information regarding general precautions when using culture media.

Refer to the keyword "MSDS", in the Hardy Diagnostics' software program HUGO™, for more information on handling potentially hazardous material.

PROCEDURE

Specimen Collection: TSA with Lecithin and Tween® 80 is not recommended for primary isolation. Consult listed references for information on specimen collection.[1,2]

Method of Use: Allow medium to warm to room temperature prior to inoculation. Consult listed references for information concerning testing procedures.[1,2]

For melting bottled and tubed media: Autoclave at 121 degrees C. for 1-3 minutes or until melted. Alternatively, a covered, boiling waterbath (100 degrees C.) can be used. There should be enough water in the waterbath to reach the media line. A covered waterbath will help to reach and maintain the temperature. Heat in waterbath until melted. Cool media to 45-50 degrees C. and dispense as desired.

Contact Plate Method:

1. Hold the contact plate with thumb and second finger and use index finger to press plate bottom firmly against the selected test surface. The same amount of pressure should be applied for every sample. Do not move plate laterally. Lateral movement spreads contaminants over the agar surface, thus making resolution of colonies difficult. A rolling motion may be used for slightly curved surfaces.[1]

2. Incubate the plates aerobically at 35 degrees C. for 48 hours. Using adequate light and magnification, count the number of colonies within the squares of the grid area. Take care not to count a square more than once.

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 entire agar surface.

4. Incubate plates at 35 degrees C. for 48 hours.

Pour Plate Method:

1. Melt agar by placing in a boiling waterbath until liquified.

2. Cool media to 45-50 degrees C. Maintain in a 45-50 degree waterbath until ready to pour.

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

4. Place a 1ml inoculum into a sterile petri plate.

5. Aseptically pour approximately 18ml of the cooled media (45-50 degrees C.) over the inoculum. Carefully swirl the plate to mix the inoculum evenly.

Note: Do not heat media longer than 3 hours at 45-50 degrees C. Sterile solidified medium can only be remelted once.

6. Allow plate(s) from step 5 to solidify.

7. Incubate plates aerobically at 35 degrees C. for 48 hours.

Data should be collected and recorded according to a designed monitoring system that statistically provides for the accurate acquisition of data.

INTERPRETATION OF RESULTS

Similar appearing colonies growing in close proximity, but not touching, should be counted as individual colonies. Colonies with different morphology or color should be counted as individual colonies. When spreading colonies are present, a representative portion of colonies in a spread-free area should be counted. This is only done if the area covered by the spreaders does not exceed one-half of the plate area.

Consult listed references for more detailed information concerning plate count methods.[1,2]

LIMITATIONS

It is recommended that biochemical and/or serological tests be performed on colonies from pure culture for complete identification.

Accurate counting can be made difficult by molds or spreading colonies.

Results can be uninterpretable or misleading unless a statistical method for monitoring is designed.

Sampling challenges may occur as a result of irregular, porous, rough or textured media surface.

Microbial contamination on a surface cannot be completely characterized by a single assay.

Refer to the keyword "Limitations", in the Hardy Diagnostics software program HUGO™, for more information regarding general limitations on culture media.

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

The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Staphylococcus aureus ATCC® 25923	A	24hrs	35°C	Aerobic	Growth
Aspergillus brasiliensis ATCC® 16404	G	1-5 days	20-25°C	Aerobic	Growth
Escherichia coli ATCC® 25922	A	24hrs	35°C	Aerobic	Growth

* Refer to the keyword "Inoculation Procedures", in the Hardy Diagnostics' software program HUGO™, for a description of inoculation procedures.

USER QUALITY CONTROL

Check for signs of contamination and deterioration. Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate

growth or a positive reaction; and at least one organism to demonstrate inhibition or a negative reaction (where applicable). Refer to the following keywords, in the Hardy Diagnostics' software program HUGO™, for more information on QC: "Introduction to QC", "QC of Finished Product", and "The CLSI (NCCLS) Standard and Recommendations for User QC of Media". Also see listed references for more information.[1.2]

Physical Appearance

TSA with Lecithin and Tween® 80 should appear clear to slightly hazy, and light amber in color.

REFERENCES

1. American Public Health Association. 2012. *Standard Methods for the Examination of Water and Wastewater,* 22nd ed. APHA, Washington, D.C.

2. APHA Technical Committee on Microbiological Methods for Foods. 2001. *Compendium of Methods for the Microbiological Examination of Foods,* 4th ed. APHA, Washington, D.C.

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