IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

TRYPTIC SOY AGAR (TSA), USP

Cat. no. G60	TSA, USP, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. G60BX	TSA, USP, 15x100mm Plate, 18ml	100 plates/box
Cat. no. J330	TSA / CET (Cetrimide Selective Agar) / MSA (Mannitol Salt Agar), USP, 15x100mm Triplate, 7ml/section	10 plates/bag
Cat. no. H19	TSA, USP, 15x150mm Plate, 69ml	10 plates/bag
Cat. no. W64	TSA, USP, 15x100mm Plate, 26ml	10 plates/bag
Cat. no. Q58	TSA, USP, 20x125mm Tube, 18ml Deep	20 tubes/box
Cat. no. Q80	TSA 1.5x, USP, 20x150mm Tube, 20ml Deep	100 tubes/box
Cat. no. Q85	TSA, USP, 20x150mm Glass Tube, 20ml Deep	100 tubes/box
Cat. no. U49	TSA, USP, 8oz. Glass Bottle, 150ml	20 bottles/box
Cat. no. U158	Tryptic Soy Agar (TSA), USP, 500ml Glass Bottle, 500ml	1 each
Cat. no. U260	TSA, USP, 8oz. Glass Bottle, 200ml	12 bottles/box
Cat. no. U360	TSA, USP, 16oz. Glass Bottle, 400ml	12 bottles/box
Cat. no. U361	Tryptic Soy Agar (TSA), USP, 500ml Polycarbonate Bottle, 500ml	10 bottles/box
Cat. no. U373	Tryptic Soy Agar (TSA), USP, 1L Polycarbonate Bottle, 1000ml	10 bottles/box

INTENDED USE

Hardy Diagnostics Tryptic Soy Agar (TSA), USP is recommended for use as a general growth medium for the detection and enumeration of microorganisms from non-clinical samples (except G60, G60BX, J330, and Q58) as specified by the United States Pharmacopoeia (USP).[(1)] In addition, the medium complies with the harmonized European, U.S. and Japanese Pharmacopoeias for determining the microbial quality of non-sterile products.[(1)]

Cat. nos. H19, W64, Q80, Q85, U49, U158, U260, U360, U361, and U373 products are not intended to be used for the diagnosis of human disease.

SUMMARY

Tryptic Soy Agar (TSA), USP is formulated in accordance with the U.S. Pharmacopeia standard formula for Soybean-Casein Digest Agar and contains digests of soybean meal and casein, which provide amino acids and other nitrogenous compounds to promote microbial growth. Sodium chloride is added to help cells maintain osmotic equilibrium. Dextrose is added as an energy source. Agar is the solidifying agent.

FORMULA

Ingredients per liter of deionized water:*

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

Final pH 7.3 +/- 0.2 at 25ºC.

In addition:

TSA 1.5X, USP (Cat. no. Q80) contains 1.5X the above ingredients.

Prepared in accordance with USP <62>.[1]

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store Cat. nos. G60, G60BX, H19, W64, and J330 at 2-8ºC away from direct light. Store Cat. nos. Q58, Q80, Q85, U49, U158, U260, U360, U361, and U373 at 2-30ºC . 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

For Cat. nos. G60, G60BX, J330, and Q58.

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 Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic 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.

For Cat. nos. H19, W64, Q80, Q85, U49, U158, U260, U360, U361, and U373.

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: The medium should be warmed to room temperature and the surface dry prior to inoculating. 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 enforced to reduce the likelihood of accidental contamination.(1) Use of stringent aseptic techniques, appropriate sporicidal agents, and a laminar clean bench are recommended in accordance with *USP Microbiological Best Laboratory Practices <1117>* and *Sterility Testing - Validation of Isolator Systems <1208>*.

Environmental Monitoring: Consult *USP Microbiological Control and Monitoring of Aseptic Processing Environments <1116>*.(1)

Sedimentation (Settling) Plate Method: Place the plate on a clean piece of paper 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.

For re-melting solid tube and bottle media: Autoclave containers with slightly loose caps at 121ºC for 1-3 minutes or until melted. Do not heat media longer than 3 hours at 45-50ºC. Alternatively, solid agar in capped containers can be racked and placed in a covered, boiling water bath (100ºC) before use. There should be enough water in the water bath to reach the top of the media line. A covered water bath will maintain consistent temperature of the media until melted. Cool media to 45-50ºC and aseptically dispense into sterile containers. **Note:** Sterile solidified media can be re-melted only once. In addition, the use of microwaves to melt media is not advised.

Performance Testing and Preparation of Test Strains: Use stable standardized suspensions of test strains per reference method. Use appropriate diluent for making test suspensions and use suspensions within the specified time period or maintain under appropriate storage practices.(1)

Testing Growth Promotion or Inhibitory Properties of Media:	
Growth Promotion, Liquid Media	Inoculate a portion of the appropriate medium with a small number (not more than 100cfu) of the appropriate test microorganism.
Growth Promotion, Solid	Perform surface spread or plate-count methods, inoculating each plate with a small number (not

Media	more than 100cfu) of the appropriate microorganism.
Inhibitory Properties, Liquid/Solid Media	Inoculate the appropriate medium with at least 100cfu of the appropriate test microorganism.
Indicative Properties	Perform surface spread or plate-count methods, inoculating each plate with a small number (not more than 100cfu) of the appropriate microorganism.

Perform membrane filtration or the plate count method, as required.[1]

Incubate media using appropriate atmospheric, temperature, and duration conditions as outlined by the test or reference method.[1] Place plates in an inverted position until growth is evident. Incubate bacterial cultures at 30-35ºC for up to 3 days; for fungal cultures, incubate at 20-25ºC for up to 5 days.

INTERPRETATION OF RESULTS

Clearly visible growth in the form of colonies constitutes a positive result. Note the inoculum dilution with the smallest and largest quantity of growth and determine the probable number of bacterial cells per gram or milliliter of sample. Growth of the microorganism should not differ by a factor greater than two from the calculated value for a standard inoculum and should be comparable to that obtained from a previously tested and approved batch of the same medium.[1]

For environmental monitoring procedures, consult USP <1116> and count the number of colonies: report as the number of colony forming units (CFU).

For growth promotion, enumeration, or sterility testing procedures, consult USP <61>, <62>, or <71>.

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]

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.

Accurate counting may be difficult with molds or spreading colonies.

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

Rare, fastidious microorganisms may not grow on general non-selective media formulations.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, incinerators, 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
Pseudomonas paraeruginosa ATCC® 9027	J	1-3 days	30-35°C	Aerobic	Growth
Bacillus spizizenii ATCC® 6633	J	1-3 days	30-35°C	Aerobic	Growth
Candida albicans ATCC® 10231	J	1-5 days	30-35°C	Aerobic	Growth
Aspergillus brasiliensis ATCC® 16404	J	1-5 days	30-35°C	Aerobic	Growth
In addition to the above, Cat. no. G60, G60BX, and H19 also include the following:					
Staphylococcus aureus ATCC® 25923	A	18-24 hrs	35°C	Aerobic	Growth
Escherichia coli ATCC® 25922	A	18-24 hrs	35°C	Aerobic	Growth
Escherichia coli ATCC® 8739	J	1-3 days	30-35°C	Aerobic	Growth
In addition to the first group above, Cat. nos. Q85 and U361 also include the following:					
Escherichia coli ATCC® 8739	J	1-3 days	30-35°C	Aerobic	Growth
Salmonella enterica ATCC® 14028	J	1-3 days	30-35°C	Aerobic	Growth

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

Tested in accordance with USP <61>.[1]

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), USP should appear translucent, and light amber in color.

Uninoculated plate of Tryptic Soy Agar, USP (Cat. no. G60).

REFERENCES

1. *United States Pharmacopoeia and National Formulary* (USP-NF). Rockville, MD: United States Pharmacopeial Convention.

Microbiological Tests
<61> Microbial Examination of Nonsterile Products: Microbial Enumeration Tests
<62> Microbial Examination of Nonsterile Products: Tests for Specified Microorganisms
<71> Sterility Tests

General Information
<1116> Microbiological Control and Monitoring of Aseptic Processing Environments
<1117> Microbiological Best Laboratory Practices
<1208> Sterility Testing - Validation of Isolator Systems

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Ordering Information

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

HDQA 2207H [D]