

Trichophyton Agars 1 – 7

Intended Use

Trichophyton Agars are differential media used in the presumptive identification of *Trichophyton* species based on nutritional requirements.

Summary and Explanation

Members of the genus *Trichophyton* have specific nutritional requirements that are essential for definitive identification.¹⁻³ Georg and Camp devised a set of chemically-defined media for differentiation and identification of *Trichophyton* isolates based on specific vitamin and amino acid requirements.⁴ These requirements are determined by comparing growth in a basal medium (Trichophyton Agar 1 or 6) with the amount of growth obtained by providing a specific nutrient. Trichophyton Agar 2, 3 and 4 are used with medium 1 to determine whether an

isolate requires inositol, thiamine or both. Trichophyton Agar 5, equivalent to Trichophyton Agar 1 with added nicotinic acid (2 mg/L), is used with medium 1 to determine the requirement for nicotinic acid, and medium 7 is used with medium 6 to determine the requirement for histidine.

Principles of the Procedure

Nutritional requirements are determined by inoculating a control medium and a medium enriched with a specific vitamin or amino acid with *Trichophyton* isolates that have been presumptively identified by gross colony characteristics and microscopic morphology.¹⁻⁶ Moderate to heavy growth in the vitamin- or amino acid-enriched medium compared to little or no growth in the basal medium indicates that the isolate requires that nutrient.

User Quality Control

Identity Specifications

Difco™ Trichophyton Agars 1, 2, 3, 4, 6 or 7

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	5.9% solution, soluble in purified water upon boiling. Solution is light to medium amber, slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 5.9% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Trichophyton Agars 1, 2 or 3

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC™	RECOVERY AGARS 1 & 2	RECOVERY AGAR 3
<i>Trichophyton concentricum</i>	9358	Good	Good
<i>Trichophyton schoenleinii</i>	4822	Good	Good
<i>Trichophyton verrucosum</i>	34470	None to poor	Good

Difco™ Trichophyton Agar 4

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC™	RECOVERY
<i>Trichophyton rubrum</i>	28188	Good
<i>Trichophyton verrucosum</i>	34470	Poor
<i>Trichophyton violaceum</i>	8376	Good

Difco™ Trichophyton Agars 6 or 7

Prepare Trichophyton Agar 6 per label directions, plain and with 0.03 g/L L-Histidine HCl. Prepare Trichophyton Agar 7 per label directions. Inoculate with fresh cultures and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC™	RECOVERY AGAR 6 PLAIN	RECOVERY AGAR 6 WITH L-HISTIDINE HCL	RECOVERY AGAR 7
<i>Microsporium gallinae</i>	22243	Good	Good	Good
<i>Trichophyton megninii</i>	12106	Poor	Good	Good



Formulae

Difco™ Trichophyton Agar 1

Approximate Formula* Per Liter	
Vitamin Assay Casamino Acids	2.5 g
Dextrose	40.0 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Agar	15.0 g

Difco™ Trichophyton Agar 2

Approximate Formula* Per Liter	
Vitamin Assay Casamino Acids	2.5 g
Dextrose	40.0 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Agar	15.0 g
Inositol	50.0 mg

Difco™ Trichophyton Agar 3

Approximate Formula* Per Liter	
Vitamin Assay Casamino Acids	2.5 g
Dextrose	40.0 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Agar	15.0 g
Inositol	50.0 mg
Thiamine HCl	200.0 µg

Difco™ Trichophyton Agar 4

Approximate Formula* Per Liter	
Vitamin Assay Casamino Acids	2.5 g
Dextrose	40.0 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Agar	15.0 g
Thiamine HCl	200.0 µg

Difco™ Trichophyton Agar 6

Approximate Formula* Per Liter	
Ammonium Nitrate	1.5 g
Dextrose	40.0 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Agar	15.0 g

Difco™ Trichophyton Agar 7

Approximate Formula* Per Liter	
Ammonium Nitrate	1.5 g
Histidine HCl	30.0 mg
Dextrose	40.0 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Agar	15.0 g

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

Directions for Preparation from Dehydrated Product

Difco™ Trichophyton Agars 1, 2, 3, 4, 6 and 7

1. Suspend 59 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 12 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Using a sterile inoculating loop or needle, remove a small amount of colony growth from the isolation medium and streak the agar surface. A small inoculum should be used to prevent carry-over of essential nutrients from the isolation medium.

Incubate medium at room temperature for up to 2 weeks.

Expected Results

Record the amount of growth using + to indicate a trace of submerged growth to 4+ to indicate maximum growth. Consult appropriate texts for information needed for interpretation of the results.^{1,2}

Limitations of the Procedure

1. It is important that pure cultures from a medium that is not vitamin enriched, such as Sabouraud Dextrose Agar or another general-purpose fungal medium, be used for the inoculum.
2. If cultures are contaminated with bacteria, the cultures should be grown on a fungal medium containing antibiotics for several generations to eliminate the bacteria. Many bacteria synthesize vitamins and may invalidate the test results.
3. When inoculating Trichophyton Agars, take care not to carry-over growth substances from primary cultures to the tube media used in the differential tests. Inocula transferred to the nutrition tubes should be very small.

References

1. Roberts. 1985. *In* Washington (ed.), Laboratory procedures in clinical microbiology, 2nd ed. Springer-Verlag, New York, N.Y.
2. Weitzman, Rosenthal and Silva-Hutner. 1988. Superficial and cutaneous infections caused by molds: dermatomycoses. *In* Wentworth (ed.), Diagnostic procedures for mycotic and parasitic infections, 7th ed. American Public Health Association, Washington, D.C.
3. Kane and Summerbell. 1999. *In* Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
4. Georg and Camp. 1957. *J. Bacteriol.* 74:113.
5. Haley, Transdel and Coyle. 1980. Cumitech 11, Practical methods for culture and identification of fungi in the clinical mycology laboratory. Coord. ed., Sherris. American Society for Microbiology, Washington, D.C.
6. McGinnis and Pasarell. 1992. *In* Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Trichophyton Agar 1

Cat. No. 287710 Dehydrated – 500 g

BBL™ Trichophyton Agar 1

Cat. No. 296243 Prepared Slants (C Tubes) – Pkg of 10*

Difco™ Trichophyton Agar 2

Cat. No. 287410 Dehydrated – 500 g

BBL™ Trichophyton Agar 2

Cat. No. 296244 Prepared Slants (C Tubes) – Pkg. of 10*

Difco™ Trichophyton Agar 3

Cat. No. 296510 Dehydrated – 500 g

BBL™ Trichophyton Agar 3

Cat. No. 296245 Prepared Slants (C Tubes) – Pkg. of 10*

Difco™ Trichophyton Agar 4

Cat. No. 219710 Dehydrated – 500 g

BBL™ Trichophyton Agar 4

Cat. No. 296246 Prepared Slants (C Tubes) – Pkg. of 10*