HC Agar Base

Intended Use

HC Agar Base, when supplemented with Polysorbate 80, is used for enumerating molds in cosmetic products.

Summary and Explanation

Methods for isolating molds from cosmetic products require incubation for 5 to 7 days using traditional agar media.[1] In 1986, Mead and O'Neill[2] described a new medium, HC Agar, for enumerating molds in cosmetic products that decreased incubation time to 3 days at 27.5 ± 0.5°C. HC Agar Base, based on the HC Agar formula of Mead and O'Neill, is supplemented with Polysorbate 80 to prepare HC Agar.

Principles of the Procedure

HC Agar Base contains peptones as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose provides a source of fermentable carbohydrate. Ammonium chloride and magnesium sulfate provide essential ions. Disodium and monopotassium phosphates buffer the pH to near neutrality. Sodium carbonate inactivates low levels of preservatives that are active at a more acidic pH (e.g., benzoic acid). Chloramphenicol inhibits bacteria, including *Pseudomonas aeruginosa* and *Serratia marcescens*, that are potential contaminants of cosmetic products. Polysorbate 80 neutralizes preservatives and sequesters surfactants that may be present in residual amounts from the product sample.[2] Agar is the solidifying agent.

Formula

Difco™ HC Agar Base

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	2.5	g
Proteose Peptone	2.5	g
Yeast Extract	5.0	g
Dextrose	20.0	g
Disodium Phosphate	3.5	g
Monopotassium Phosphate	3.4	g
Ammonium Chloride	1.4	g
Magnesium Sulfate	0.06	g
Chloramphenicol	0.1	g
Sodium Carbonate	1.0	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 54.5 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. Add 20 mL of Polysorbate 80.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ HC Agar Base

Dehydrated Appearance:	Very light to light beige, free-flowing, homogeneous.
Solution:	5.45% solution, soluble in purified water upon boiling. Solution is medium to dark amber, slightly opalescent to opalescent, may have a slight precipitate.
Prepared Appearance:	Medium amber with yellow tint, very slightly to slightly opalescent, no significant precipitate.
Reaction of 5.45% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ HC Agar Base

Prepare the medium per label directions. Inoculate and incubate at 27.5 ± 0.5°C for 65-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus brasiliensis (niger)	16404	10^2-10^3	Good
Pseudomonas aeruginosa	10145	10^3-2×10^3	None to poor
Serratia marcescens	13880	10^3-2×10^3	None to poor

Procedure

1. Process each specimen as appropriate for that specimen and inoculate directly onto the surface of the medium.[1] Inoculate duplicate plates.
2. Incubate plates aerobically at 27.5 ± 0.5°C.
3. Examine plates for growth and recovery after 72 hours of incubation.
4. Count mold colonies from duplicate plates and record average count as mold count per gram or milliliter of sample.

Expected Results

Mold cultures should yield good growth and recovery. Bacteria should be inhibited.

Limitation of the Procedure

The 27.5 ± 0.5°C incubation temperature is critical for obtaining statistically significant mold counts after three days using this medium.

References

1. U.S. Food and Drug Administration. 2001. FDA bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
2. Mead and O'Neill. 1986. J. Soc. Cosmet. Chem. 37:49.

Availability

Difco™ HC Agar Base

Cat. No. 268510 Dehydrated – 500 g