

HC AGAR BASE (7520)

Intended Use

HC Agar Base is used with Tween 80 (Polysorbate 80) for the enumeration of molds in cosmetics.

Product Summary and Explanation

Methods for isolating molds from cosmetic products require incubation for 5 - 7 days using traditional agar media.¹ In 1986, Mead and O'Neill described a new medium, HC Agar, for enumerating molds in cosmetic products.² The formulation of HC Agar decreased incubation time to 3 days at $27.5 \pm 0.5^\circ\text{C}$ for molds.² HC Agar Base, based on the HC Agar formula of Mead and O'Neill, is supplemented with Tween 80 (Polysorbate 80) to prepare HC Agar.²

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, amino acids, and carbon in HC Agar Base. Yeast Extract is a vitamin source required for organism growth. Dextrose is the fermentable carbohydrate. The Phosphates buffer the pH to near neutrality. Ammonium Chloride and Magnesium Sulfate provide essential ions. Sodium Carbonate inactivates low levels of preservatives that are active at an acidic pH. Chloramphenicol inhibits bacteria, including *Pseudomonas aeruginosa* and *Serratia marcescens*, potential contaminants of cosmetic products. Tween 80 (Polysorbate 80) neutralizes preservatives and sequesters surfactants that may be present in residual amounts from product samples.² Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	2.5 g
Enzymatic Digest of Animal Tissue.....	2.5 g
Yeast Extract.....	5 g
Dextrose.....	20 g
Disodium Phosphate.....	3.5 g
Monopotassium Phosphate	3.4 g
Ammonium Chloride	1.4 g
Sodium Carbonate	1 g
Magnesium Sulfate	0.06 g
Chloramphenicol.....	0.1 g
Agar	15 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement / Liter (7992)

Tween 80, 20 mL

Precautions

1. For Laboratory Use.
2. TOXIC. Toxic if swallowed, inhaled, or absorbed through the skin. Irritating to skin, eyes, and respiratory system. Possible risk of harm to unborn child.

Directions

1. Suspend 54.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Add 20 mL of Tween 80 (Polysorbate 80) and mix.
4. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light to medium beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and medium to dark amber.

Expected **Cultural Response:** Cultural response on HC Agar Base supplemented with Tween 80 incubated aerobically at $27 \pm 0.5^\circ\text{C}$ and examined for growth after 2 - 7 days incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Aspergillus niger</i> ATCC® 16404	Point Inoculation	Growth
<i>Bacillus subtilis</i> ATCC® 9372	300 - 1000	Inhibited
<i>Candida albicans</i> ATCC® 10231	10 - 300	Fair to excellent
<i>Escherichia coli</i> ATCC® 25922	300 - 1000	Inhibited
<i>Penicillium roquefortii</i> ATCC® 10110	Point Inoculation	Growth
<i>Pseudomonas aeruginosa</i> ATCC® 27853	300 - 1000	Marked to complete inhibition
<i>Staphylococcus aureus</i> ATCC® 25923	300 - 1000	Inhibited

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Process each specimen as appropriate and inoculate directly onto surface of the medium.¹ Inoculate duplicate plates.
2. Incubate plates aerobically at $27 \pm 0.5^\circ\text{C}$.
3. Examine plates for growth and recovery after 72 hours incubation.
4. Count mold colonies from duplicate plates and record average count as mold count per gram or milliliter of sample.

Results

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

Storage

Store sealed bottle containing dehydrated medium at 2 - 30°C . Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow.
2. The $27 \pm 0.5^\circ\text{C}$ incubation temperature is critical for obtaining statistically significant mold counts after three days.

Packaging

HC Agar Base	Code No.	7520A	500 g
		7520B	2 kg
		7520C	10 kg
Tween 80		7992	100 mL

References

1. Hitchins, A. D., T. T. Tran, and J. E. McCarron. 1995. Microbiological methods in cosmetics. In Bacteriological analytical manual, 8th ed AOAC International, Gaithersburg, MD.
2. Mead, C., and J. O'Neill. 1986. A three-day mold assay for cosmetics and toiletries. J. Soc. Cosmet. Chem. **37**:49-57.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.

