HC Agar Base (NCM0155)

Intended Use
HC Agar Base is used with Tween 80 (Polysorbate 80) for the enumeration of molds in cosmetics in a laboratory setting. HC Agar Base is not intended for use in the diagnosis of disease or other conditions in humans.

Description
Methods for isolating molds from cosmetic products require incubation for 5 - 7 days using traditional agar media. In 1986, Mead and O'Neill described a new medium, HC Agar, for enumerating molds in cosmetic products. The formulation of HC Agar decreased incubation time to 3 days at 27.5 ± 0.5°C for molds. 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.

Typical Formulation

Enzymatic Digest of Casein	2.5 g/L
Enzymatic Digest of Animal Tissue	2.5 g/L
Yeast Extract	5.0 g/L
Dextrose	20.0 g/L
Disodium Phosphate	3.5 g/L
Monopotassium Phosphate	3.4 g/L
Ammonium Chloride	1.4 g/L
Sodium Carbonate	1.0 g/L
Magnesium Sulfate	0.06 g/L
Chloramphenicol	0.1 g/L
Agar	15.0 g/L

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

Precaution
Refer to SDS

Preparation
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 (7992) and mix.
4. Autoclave at 121°C for 15 minutes.
5. Cool to 45-50°C.

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.

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Expected Cultural Response: Cultural response on HC Agar Base supplemented with Tween 80 incubated aerobically at 27 ± 0.5°C and examined for growth after 2 - 7 days incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results
Aspergillus brasiliensis ATCC® 16404	Point Inoculation	Growth
Bacillus subtilis ATCC® 9372	300 - 1000	Completely Inhibited
Candida albicans ATCC® 10231	10 - 300	Fair to Excellent
Escherichia coli ATCC® 25922	300 - 1000	Completely Inhibited
Penicillium roquefortii ATCC® 10110	Point Inoculation	Growth
Pseudomonas aeruginosa ATCC® 27853	300 - 1000	Partially to Completely Inhibited
Staphylococcus aureus ATCC® 25923	300 - 1000	Completely 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. Inoculate duplicate plates.
2. Incubate plates aerobically at 27 ± 0.5°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.

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 ± 0.5°C incubation temperature is critical for obtaining statistically significant mold counts after three days.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. 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.

References

1. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalytical manualBAM/ default.htm.
2. Mead, C., and J. O'Neill. 1986. A three-day mold assay for cosmetics and toiletries. J. Soc. Cosmet. Chem. 37:49-57.

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