

Application

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Efficacy Tests for ProClin Preservative

The two tests described here will enable you to estimate the ability of ProClin preservative to protect your product. Use the Protection Testing Procedure to determine the level of ProClin preservative needed to prevent microbial growth. Two levels of the preservative generally are tested, with the levels selected based on application needs, such as the time period over which preservation is required (e.g., 1 week or 1 year), how harsh the product environment is toward the preservative (e.g., pH, amine or reducing agent content), and the type and level of microbial contamination the product may encounter in manufacturing, storage, or use. Use the Preservative Stability Test to determine the stability of ProClin preservative in your product, to estimate how long the preservative will be effective.

Key Words:

- ProClin preservative

Protection Testing Procedure

1. Treat samples of the product with the test level(s) of ProClin® preservative. Reserve an aliquot of unpreserved product as a control, to demonstrate the ability of the product to support microbial growth.
2. Inoculate the preservative-treated and control samples with a high level of inoculum (10^6 – 10^7 total colony forming units/mL of sample). Prepare the inoculum mixture from among the organisms listed in Table 1 or, for a truer evaluation of performance, use the microbe(s) most likely to occur in your product (e.g., by inoculating samples with naturally contaminated product).
3. Incubate the inoculated samples at 25°C for two weeks.
4. Determine microbe levels in the samples by streaking an aliquot of each sample onto a tryptic soy broth agar (TSBA) plate, incubating the plates at 30°C for one week, then examining and rating them as indicated in Table 2.
5. Inoculate each sample a second time, incubate at 25°C for another two weeks, and streak onto TSBA plates. Incubate the plates at 30°C for one week, then examine and rate them as described in step 4. A ProClin preservative level is deemed effective if no microbial growth (<10 CFU/mL) is detected after each inoculation.

To perform a simulated accelerated aging study, treat a second set of samples with the preservative and place them in a dry bath at 45°C. After four weeks, cool the samples to ambient temperature, then inoculate, incubate, plate, and examine, as described in steps 2–4. Substitute other aging conditions to address specific needs.



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Table 1. Inoculum for Evaluating Protection by ProClin Preservative

Microorganism	ATCC No.*
Gram-Negative Bacteria	
<i>Pseudomonas aeruginosa</i>	15442
<i>Pseudomonas cepacia</i>	17765
Gram-Positive Bacterium	
<i>Staphylococcus aureus</i>	6538
Yeast	
<i>Candida albicans</i>	11651
Fungus	
<i>Aspergillus niger</i>	6275

*American Type Culture Collection

Table 2. Rating Streaked Plates for Colony-Forming Units (CFU)

Score Assigned to Plate	Estimated CFU/mL
0	<10
T (trace)	10 – <100
1+	100 – <10 ³
2+	10 ³ – <10 ⁴
3+	10 ⁴ – <10 ⁵
4+	≥10 ⁵

Preservative Stability Test

This test typically is performed on uninoculated samples.

1. Treat samples of the product with the test level(s) of ProClin preservative. Divide each sample into two vials. Store one vial in an incubator at 25°C; store the second in a dry bath at 45°C.
2. Analyze the samples at various time intervals after introducing the preservative, using the HPLC methods described in Rohm and Haas Technical Bulletin CS-604. A typical analysis schedule is: immediately after introduction of the preservative, after two weeks and after four weeks at 45°C, and after 4 weeks at 25°C. Other storage temperatures and analysis schedules can be substituted to address specific needs. Any significant loss of the active agent, RH-651 (i.e., >20%), over the test period, under the conditions you choose, is a clear indication that additional work should be done to determine and/or improve stability.

Note that while the stability test results are an indicator of the length of time ProClin preservative will effectively preserve the product, a specific shelf life cannot be extrapolated from these limited results.

Ordering Information:

ProClin Preservative

Please refer to the product specification sheets in the ProClin literature packet. If you do not have the packet, request it from our Technical Service group (phone: 800-359-3041 or 814-359-3041, fax: 814-359-5468).

Microbe Cultures

Contact: American Type Culture Collection
Rockville, MD 02060 USA
Customer service/ordering: 800-638-6597
Main office: 301-881-2600

Trademark

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Contact our Technical Service Department
(phone 800-359-3041 or 814-359-3041, FAX 800-359-3044 or
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for expert answers to your questions.

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