Instituto Valenciano de Microbiología

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Paints and varnishes - Testing the efficacy against fungi of film preservatives in a coating with the product "Protox Skimmel"
(EN 15457: 2022 Standard)

Report
Registration No.: D/24/B0372.

1. **Laboratory identification** ……. Instituto Valenciano de Microbiología

2. **Client identification** ……..……. Protox ApS.
   Address …….…….. Fabriksvej 19
   DK-6000 Kolding.

3. **Sample identification** (information provided by the client)

- Product name .....……. **Protox Skimmel.**
- Batch number ..…… 67.59.
- Control product name ……..……..... **Protox Skimmel – without IPBC.**
- Batch number ……. Not applicable.
- Expiration date …….…….…….. 2 years after production date: production date 21-03-2024.
- Manufacturer /supplier …..…..…….…… Protox ApS.
- Storing conditions .....……. Frost free.
- Active(s) substance(s) and its concentration(s) …….. 3-Iodo-2-Propynylbutylcarbamate 9 g/L.
- Product application method …… Applied by brush or roll.

IVAMI is not responsible for client-supplied information.

4. Information about sample reception

• Date of reception of the sample ……..	2024/06/07.
• Date of reception of test conditions	2024/06/10.
• Aspect of the received sample (describe if any alterations are found in the packaging or in the test sample: leaks, layer separation, surface films, impurities, etc.) …….....	Milky white liquid in plastic bottle. No alteration is observed in the sample.
• Aspect of the received control sample (describe if any alterations are found in the packaging or in the test sample: leaks, layer separation, surface films, impurities, etc.) …….....	Milky white liquid in plastic bottle identified without IPBC. No alteration is observed in the control sample.

5. Method of assay

This test is performed following the indications of **EN 15457: 2022** Standard**.**

6. Experimental conditions

• Period of analysis (including strain preparation).	2024/09/18 to 2024/10/16.
• Substrate on which the test sample is applied	Filter paper without fungicidal effect.
• Preliminary preparation of the received sample .	Homogenization.
• Method of application of the test coating (including number of layers and waiting times)….	Product, both sample and control, is applied with a brush. Dose 0.25 liter per m^2. The film is allowed to dry for a minimum of 8 hours at 20ºC.
• Method of sterilization of the specimens with test/substrate coating …….…….	The filter paper without fungicidal effect is autoclaved, without application of the product.
• Conditioning of test specimens ……..	Conditioning in horizontal position for 5 days at 23ºC ± 2ºC and 50 ± 5% relative humidity.
• Dimensions of test specimens ……	55 mm diameter.
• Number of specimens tested …….	3 specimens with biocide coating, 3 specimens with coating without biocide and 3 specimens of the uncoated substrate.
• Volume of spore suspension inoculated onto the specimens ……..…….	0.2 mL.

- Incubation conditions of the specimens on the agar plates …… 24ºC ± 2ºC.
- Periods for assessment fungal growth …… 7 and 14 days after inoculation.

- Identification of test strains:

    - Fungi more likely to grow in an indoor environment:
        - *Aspergillus niger* (DSM 12634).
        - *Stachybotrys chartarum* (IMI 082021 = DSM 2144).

    - Fungi more likely to grow in an external environment:
        - *Alternaria alternata* (DSM 62010).
        - *Cladosporium cladosporioides* (DSM 62121).

7. Results of the assay

- Validation tests and controls …..... See tables 1 to 5.
- Evaluation of fungicidal activity..... See table 6.
- Method for evaluating fungal growth …… See table 7

8. Remarks

- All controls and validation were within their basic limits.
- The maximum duration of the assay must be 21 days. However, the test can be considered to have been completed at an earlier stage if the non-biocide-coated specimens have a score of 4.
- The test is terminated 14 days after inoculation since it is observed that the non-biocide-coated specimens have a score of 4.
- The efficacy of film preservatives in the coating is demonstrated if the test specimens containing film preservatives are rated less than 4 (see table 7).

9. Conclusion

The biocide coating product **Protox Skimmel** batch 67.59 **is effective** against the test strains *Alternaria alternata* (DSM 62010), *Cladosporium cladosporioides* (DSM 62121), *Aspergillus niger* (DSM 12634) and *Stachybotrys chartarum* (IMI 082021 = DSM 2144) with a rating of 0, after an incubation time of 14 days, when evaluated according to the **EN 15457: 2022** Standard**.**

Note: The results obtained correspond to the sample received in the laboratory.

Bétera (Valencia), October 21, 2024

MONTOYA VIECO, ELENA (FIRMA)

Signed. Elena Montoya
Responsible Technician

ESTEBAN BERMUDEZ, ENCARNACION PILAR (FIRMA)

Signed. Encarnación Esteban
Technical Director

Reference

- **EN 15457: 2022**- Paints and varnishes - Laboratory method for testing the efficacy of film preservatives in a coating against fungi.

Results of the assay - Fungicidal efficacy

Table 1.- Suspension of the test (*N*) of the fungus *Aspergillus niger* (DSM 12634).

Seeding: Pour plate; Number of plates: 4 /mL.

	N	***V_{C1}***	***V_{C2}***	
Suspension of the test (***N***)	10^{-6}	11	12	***Xwm*** = 8.80 x 10^6,
	10^{-5}	92	84	10^6 CFU/mL ≥ *N* ≤ 10^7 CFU/mL? Yes

Plate counts
N (10^{-5}): 23 + 19 + 25 + 25; 18 + 23 + 21 + 22;
(10^{-6}): 3 + 2 + 4 + 2; 5 + 2 + 3 + 2;

Table 2.- Suspension of the test (*N*) of the fungus *Stachybotrys chartarum* (IMI 082021 = DSM 2144).

Seeding: Pour plate; Number of plates: 4 /mL.

	N	***V_{C1}***	***V_{C2}***	
Suspension of the test (***N***)	10^{-6}	15	16	***Xwm*** = 9.77 x 10^6,
	10^{-5}	90	94	10^6 CFU/mL ≥ ***N*** ≤ 10^7 CFU/mL? Yes

Plate counts
N (10^{-5}): 20 + 22 + 19 + 29; 24 + 26 + 21 + 23;
(10^{-6}): 3 + 4 + 2 + 6; 2 + 5 + 3 + 6;

Table 3.- Suspension of the test (*N*) of the fungus *Alternaria alternata* (DSM 62010).

Seeding: Pour plate; Number of plates: 4 /mL.

	N	***V_{C1}***	***V_{C2}***	
Suspension of the test (***N***)	10^{-6}	10	12	***Xwm*** = 7.60 x 10^6,
	10^{-5}	80	72	10^6 CFU/mL ≥ ***N*** ≤ 10^7 CFU/mL? Yes

Plate counts
N (10^{-5}): 18 + 21 + 23 + 18; 17 + 15 + 19 + 21;
(10^{-6}): 4 + 3 + 2 + 1; 2 + 5 + 3 + 2;

Table 4.- Suspension of the test (*N*) of the fungus *Cladosporium cladosporioides* (DSM 62121).

Seeding: Pour plate; Number of plates: 4 /mL.

	N	***V_{C1}***	***V_{C2}***	
Suspension of the test (***N***)	10^{-6}	9	10	***Xwm*** = 6.60 x 10^6,
	10^{-5}	68	64	10^6 CFU/mL ≥ ***N*** ≤ 10^7 CFU/mL? Yes

Plate counts
N (10^{-5}): 16 + 15 + 20 + 17; 17 + 15 + 16 + 16;
(10^{-6}): 2 + 3 + 2 + 2; 4 + 2 + 3 + 1;

Table 5.- Evaluation of fungal growth in test tubes and control plates

Controls	**Plate growth control: plates with inoculated culture medium**			**Growth control on the substrate: Uncoated specimens**			**Growth control in the coating: Coated specimens without biocide**		
	Plate 1	Plate 2	Plate 3	Specimen 1	Specimen 2	Specimen 3	Specimen 1	Specimen 2	Specimen 3
Day 7	G	G	G	G	G	G	G	G	G
Day 14	G	G	G	G	G	G	G	G	G
Day 21	-	-	-	-	-	-	-	-	-

G: Detection of test fungal growth.
NG: No growth of test fungi detected.

Test validation
For the test to be considered valid:
- Growth must exist on the plates with inoculated culture medium, on the uncoated substrate test tubes and on the coated test tubes without biocide.
- In none of the three controls should the presence of other microorganisms be detected in a quantity that could interfere with the evaluation.

Table 6.- Evaluation of fungal growth on the test specimens with the biocide coating (see Table 7 to assign the grading to each specimen)

Specimen of assay	**Biocide coated specimens**		
	Specimen 1	Specimen 2	Specimen 3
Day 7	0	0	0
Day 14	0	0	0
Day 21	-	-	-

Table 7.- Classification system used to designate the percentage area of disfigurements (Table B.1 of EN 15457: 2022)

Rating	Percentage area of disfigurements
0	No growth on the surface of the test specimen.
1	Up to 10% growth on the surface of the test specimen.
2	More than 10% up to 30% growth on the surface of the test specimen.
3	More than 30% up to 50% growth on the surface of the test specimen.
4	More than 50% up to 100% growth on the surface of the test specimen.