TECHNICAL DATA SHEET

RHAPSODY AGAR®

ENUMERATION OF *PSEUDOMONAS* SPP.

1 INTENDED USE

RHAPSODY Agar® is a selective medium used for the enumeration of *Pseudomonas* spp. in food products and environmental samples.

This method is officially certified by AFNOR Certification for the enumeration of presumptive *Pseudomonas* spp. in meat products, in comparison to the reference method NF EN ISO 13720 : 2010, under the reference number BKR 23/09-05/15 A, of which the validity runs until June 5th , 2019.

BKR 23/09/-05/15 A,
METHODES ALTERNATIVES D'ANALYSE
POUR L'AGROALIMENTAIRE
Certifié par AFNOR Certification **http://nf-validation.afnor.org**

This method is officially certified by AFNOR Certification for the enumeration of *Pseudomonas* spp. in dairy products, in comparison to the reference method XP ISO/TS 11059 : 2009, under the reference number BKR 23/0905/15 B, of which the validity runs until June 5th, 2019.

BKR 23/09/-05/15 B,
METHODES ALTERNATIVES D'ANALYSE
POUR L'AGROALIMENTAIRE
Certifié par AFNOR Certification **http://nf-validation.afnor.org**

The validated method following the validation protocol EN ISO 16140 allows the enumeration in 48 ± 3 hours without confirmation for meat products and dairy products.

2 PRINCIPLES

The peptones constitute nutritive substrates necessary for the rapid growth of *Pseudomonas*.

The chromogenic substrate contained in the medium is hydrolyzed by all *Pseudomonas*. As a result, colonies present a light blue to blue-green coloration.

The selective system and the cephalosporin insure the inhibition of the majority of the secondary flora.

3 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1010 mL of complete media :

- Polypeptone ..... 28,9 g
- Buffered system ..... 7,0 g
- Sodium chloride ..... 5,0 g
- Selective system ..... 5,5 g
- Chromogenic mixture ..... 0,2 g
- Bacteriological agar ..... 15,0 g

pH of the ready-to-use media at 25°C : 7,0 ± 0,2.

4 PREPARATION

Preparation from dehydrated media :

- Dissolve 61,6 g of dehydrated media (BK214) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in vials of 100 mL.
- Sterilize in an autoclave at 110 °C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.

✓ **Reconstitution : 61,6 g/L**

✓ **Sterilization : 15 min at 110 °C**

Rehydration of freeze-dried supplement :

- Rehydrate the pellet (BS089) by adding aseptically 5 mL of sterile distilled water.
- Turn end-over-end to dissolve. Avoid frothing the solution

Preparation from complete media :

- Aseptically add 1 mL of reconstituted RHAPSODY Agar® Supplement (BS08908) to each 100 mL vial of base medium.
- Homogenize thoroughly.
- Pour the appropriate amount of complete media into sterile Petri dishes and let solidify on a cool surface.

5 INSTRUCTIONS FOR USE

Follow good laboratory practices guidelines (refer to ISO 7218 standard).

- To the surface of plates prepared as above or pre-poured plates (BM16708), transfer 0.1 mL of the sample to be tested and its serial dilution.
- Spread the inoculum on the surface with the aid of a sterile triangle or « hockey stick ».
- Incubate at 30 ± 1 °C for 48 hours ± 3 hours.

✓ **Inoculation : 0,1 mL on surface**

✓ **Incubation : 48 ± 3 h at 30 ± 1 °C**

Notes

- The method is also validated for the Spiral inoculation technique.
- The enumeration limit can be reduced by a factor of 10 by inoculating 1 mL of initial suspension on to the surface of 3 Petri dishes (Ø 90 mm).

For organizational reasons in the laboratories, the incubation is validated between 45 and 72 hours.

6 RESULTS

Characteristic colonies show light blue to blue-green coloration.
Colony diameter and color intensity may vary according to *Pseudomonas* species.
The expression of the results should be made conform to the recommendations established in ISO 7218.

Note
If the interpretation is subject to uncertainty after 48 hours (i.e. too light colonies), incubation must be prolonged to 72 hours.

See ANNEX 1 : PHOTO SUPPORT.

7 QUALITY CONTROL

Dehydrated media : beige powder, free-flowing and homogeneous.
Complete media : amber agar.

Typical culture response on complete media after 48 hours of incubation at 30 °C :

Microorganisms		Growth (Productivity Ratio : P_R)
Pseudomonas aeruginosa	WDCM 00026	$P_R \geq 50\%$
Pseudomonas aeruginosa	WDCM 00025	$P_R \geq 50\%$
Pseudomonas putida	WDCM 00117	$P_R \geq 50\%$
Pseudomonas fluorescens	WDCM 00115	$P_R \geq 50\%$
Escherichia coli	WDCM 00013	Inhibited, score 0
Staphylococcus aureus	WDCM 00034	Inhibited, score 0

8 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
Freeze-dried supplements : 2-8 °C.
Pre-poured media : 2-8 °C.
The expiration dates are indicated on the labels.

Prepared media in plates (*) : 30 days at 2-8 °C.
(*) : Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

9 PACKAGING

Pre-poured media in Petri plates (∅ 90 mm) :
20 plates BM16708

Dehydrated media
500 g bottle BK214HA

RHAPSODY Selective Supplement
10 vials qsp 500 mL BS08908

10 BIBLIOGRAPHY

XP ISO/TS 11059. Octobre 2009. Lait et produits laitiers. Méthode de dénombrement des *Pseudomonas* spp.

NF EN ISO 13720. Novembre 2010. Viandes et produits à base de viandes. Dénombrement des *Pseudomonas* spp. présomptifs.

11 ADDITIONAL INFORMATION

RHAPSODY Agar® is a registered trademark of SOLABIA S.A.S.

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning

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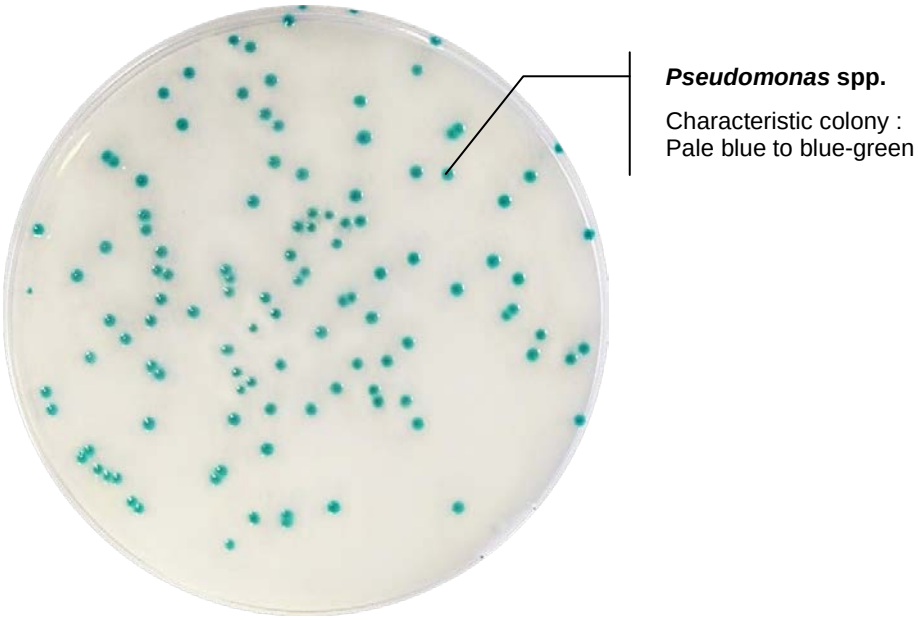
RHAPSODY Agar®

Detection and enumeration of *Pseudomonas* spp.

Results :

Growth obtained after 48 hours of incubation at 30 °C.



The size of the colonies and the intensity of the blue to blue-green coloration can vary depending on the species of *Pseudomonas* found on the plates.