

RAPID' *Campylobacter* / Agar

356-4295
356-4296

DEFINITION

RAPID' *Campylobacter* Agar is a selective chromogenic media for the detection and the enumeration of the main species of thermophilic *Campylobacter* (*C. jejuni*, *C. coli* and *C. lari*) in food products and environmental samples.

NF VALIDATION by AFNOR CERTIFICATION as per EN ISO 16140 protocol

The RAPID' *Campylobacter* method has been certified NF VALIDATION as alternative to reference method ISO/TS 10272-2 (2006) for the **enumeration of *Campylobacter*** in meat and meat products, poultry and poultry products, and environmental samples



BRD: 07/25 – 01/14
ALTERNATIVE ANALYTICAL METHODS FOR
AGRIBUSINESS
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STANDARD REFERENCES

- **ISO 10272-1:2006 (2006-01-15)**
Microbiology of food and animal feeding stuffs. Horizontal method for detection and enumeration of *Campylobacter* spp. -- Part 1: Detection method
- **ISO/TS 10272-2:2006 (2006-01-15)**
Microbiology of food and animal feeding stuffs. Horizontal method for detection and enumeration of *Campylobacter* spp. -- Part 2: Colony-count technique
- **U.S. Department of Agriculture, Food Safety and Inspection Service (2011) Microbiology Laboratory Guidebook, Chapter 41.02.**

PRINCIPLE

RAPID' *Campylobacter* is a selective chromogenic medium used for the detection of thermophilic *Campylobacter* spp. in food and environmental samples. The use of a selected nutritive mixture associated with reducing agent allows the growth of *Campylobacter* spp. in an optimal time. Other bacterial species, as well as

yeasts and molds, are inhibited by the selective agents. *Campylobacter* produce brick-red colonies on RAPID' *Campylobacter*.

PRESENTATION

Dehydrated
500g code 356-4295

Supplement Lyophilized
10 vials QSP 400 ml code 356-4296

STORAGE AND SHELF LIFE

- Dehydrated: + 15 - 25 °C, tightly closed in a cool dry place
- Supplement - Lyophilized: 2-8° safe from light.
- Medium prepared by the user:
 - Unsupplemented base: 6 weeks to 2-8°C safe from light
 - Petri dishes reconstituted: 2 weeks at 2-8°C in dark conditions packed in plastic bag or equivalent
 - Expiration date and batch number are shown on the package

THEORETICAL FORMULA

Nutritive mix	28.5 g
Reducing mix	1 g
Sodium chloride	5 g
Buffer	1.25 g
Selective mix	0.082 g
Chromogenic substract	0.05 g
Agar	14 g
Water	q.s.p. 1 L
pH (25°C) final =	7.2 - 7.5



H315: Causes skin irritation
H319: Causes serious eye irritation
H335: May cause respiratory irritation

OTHER EQUIPMENT REQUIRED NOT SUPPLIED (Non-exhaustive list)

- Balance
- Sterile weighing bags
- Grinder
- Mixer-homogenizer
- Sterile Petri dishes
- Sterile Pipettes (0.1 ml ; 1 ml...)
- inoculating loop
- Sterile Pasteur pipettes
- Water baths
- Incubator or thermostatically controlled

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containment

- All current laboratory equipment

OTHER PRODUCTS REQUIRED NOT SUPPLIED

Detection method

- Bolton broth
- Preston broth
- mCCD agar
- Appropriate system for microaerophilic culture

Enumeration method:

- Tryptone Salt :
 - 9 ml x 25 tubes (e.g.*code 355-5754)
 - 90 ml x 6 vials (e.g.*code 355-5756)
 - 500 g (e.g.*code 356-4544)
 - 5 bags 2,3 L (e.g.*code 355-5791)
- Buffered Pepton Water :
 - 6 vials 225 ml (e.g.*code 355-4179)
 - 500 g (e.g.*code 356-4684)
 - 5 bags 2,3 L (e.g.*code 355-5789)
 - 2 bags 5 L (e.g.*code 355-5790)
- Appropriate system for microaerophilic culture

Confirmation

- Columbia agar (code 356-3784)
- Physiological water (e.g.*code 355-4164)
- Oxidase test disk (e.g.*code 355-3834)
- Sterile Distilled Water (e.g.*code 355-4154)
- *Campylobacter* Confirm Latex (code 356-4297)
- iQ-Check *Campylobacter* (code 357-8135)

e.g.*: for instance

DEHYDRATED MEDIUM PREPARATION INSTRUCTIONS

Always shake bottle before each use.

Lyophilized Supplement Reconstitution (code 356-4296)

- Aseptically rehydrate the lyophilizate with 30 ml of sterile distilled water (e.g. code 355-4154).
- Stir until completely dissolved.

Complete Medium

- Dissolve 20g of powder (code 356-4295) in 370 ml of distilled water.
- Mix until a homogeneous solution.
- Heat slowly, stirring frequently, bring to a boil
- Sterilize by autoclaving for 15 min at 121°C.
- Cool to 47/50°C.
- Aseptically add 1 vial of reconstituted supplement RAPID' *Campylobacter* (code 356-4296).
- Mix well and pour in Petri dishes.
- Before inoculation, the plates must be carefully dried (see section PRECAUTIONS).

PROTOCOLS

Detection: Standard Method ISO 10272-1:2006 (2006-01-15)

Preparation and sample enrichment

- Performed in accordance with Norm ISO 10272-1:2006 (2006-01-15)

Inoculation and incubation

- Performed in accordance with Norm ISO 10272-1:2006 (2006-01-15).
- Incubate plates of RAPID' *Campylobacter*, lid down for 24 hours to 48 hours at 41.5 ± 1°C in microaerophilic atmosphere.

Reading- *Campylobacter* produce brick-red colonies

Confirmation

- Performed in accordance with Norm ISO 10272-1:2006 (2006-01-15)

Enumeration: Alternative Method validated ISO 16140

Preparation and sample enrichment, Inoculation and Incubation

Prepared samples in accordance with Norm ISO 10272-2:2006 (2006-01-15) or USDA FSIS MLG 41,02.

Spreading and Incubation

- Spread 0.1 ml on the surface of one plate of RAPID' *Campylobacter* or 1 ml onto 3 plates of RAPID' *Campylobacter*.
- If necessary, perform a dilution of 1/10 (or more) in the diluent Tryptone Salt Water or BPW in accordance with Norm ISO 6887-1 standard, and spread 0.1 ml of each dilution on 1 plate of RAPID' *Campylobacter*.
- Incubate, lid down, for 24h - 48h at 41.5 ± 1°C in a microaerophilic atmosphere.

Note: After incubation, the plates of RAPID' *Campylobacter* can be stored refrigerated (2-8°C) for 72h in a microaerophilic atmosphere before reading and/or confirmation.

Reading

Campylobacter produce brick-red colonies

Confirmation

After 24h incubation:

- Perform a PCR test (iQ-Check *Campylobacter* code 357-8135) directly from a typical colony of *Campylobacter*.

After 44 ± 4h incubation:

- According to the standard tests described in the Norm ISO 10272-1:2006 (2006-01-15) or USDA FSIS MLG 41.02.

- Perform a PCR test (iQ-Check *Campylobacter* code 357-8135) directly from a typical colony of *Campylobacter*.
- *Campylobacter* Confirm Latex (code 356-4297) on an isolated colony.

Note: According to NF VALIDATION mark, incubation time lower than 44 ± 4 h have not been validated.

Results expression

Conducted in accordance with Norm ISO 10272-2 :2006 (2006-01-15) or USDA FSIS MLG 41.02.

Note: According to the ISO 7218, confirming less than five colonies involves a risk of making an overestimation because of the presence of typical colonies that would not be *Campylobacter* spp.

Detection: Alternative Method

Sample preparation

Dilute n g or n ml of sample in $9 \times n$ ml of Preston broth or Bolton broth. Example: Dilute 25 g of sample in 225 ml of Preston broth or Bolton broth.

Enrichment

Preston : Incubate at $41.5 \pm 1^\circ\text{C}$ for 24 ± 2 h in a microaerophilic atmosphere.

Bolton : Incubate at $37 \pm 1^\circ\text{C}$ for 4 -6h then at $41.5 \pm 1^\circ\text{C}$ for 44 ± 4 h in a microaerophilic atmosphere.

Spreading and Incubation

- Collect 10 μ l enrichment broth with a sterile inoculating loop.-Inoculate RAPID' *Campylobacter* media according to traditional techniques of isolation.- Incubate RAPID' *Campylobacter*, lid down, for 24h to 44 ± 4 h at $41.5 \pm 1^\circ\text{C}$ in a microaerophilic atmosphere.

- After incubation, the plates of RAPID' *Campylobacter* can be stored refrigerated (2-8 $^\circ\text{C}$) for 72h in a microaerophilic atmosphere before reading and/or confirmation.

Reading

- *Campylobacter* produces brick-red colonies

Confirmation

After 24h of incubation:

- Perform a PCR test (iQ-Check *Campylobacter* code 357-8135) directly from a typical colony of *Campylobacter*.

After 44 ± 4 h of incubation:

- For use as described in ISO 10272-1:2006 (2006-01-15) or USDA FSIS MLG 41.02

standard conventional tests.

- Perform a PCR test (iQ-Check *Campylobacter* code 357-8135) directly from a typical colony of *Campylobacter*.
- *Campylobacter* Confirm Latex (code 356-4297) on an isolated colony.

PRECAUTIONS

- Observe Good Laboratory Practice (EN ISO 7218)
- Do not expose Petri dishes to sunlight.
- After distribution, let the Petri dishes stabilize at least 1 night at RT. Before inoculation, the plates must be sufficiently dry, with no visible surface humidity, to prevent spread of colonies (eg. by drying for 15 min at 56 $^\circ\text{C}$ in an incubator).
- The Petri dishes can be stored 2 weeks at 2-8 $^\circ\text{C}$. Before inoculation, the plates must be sufficiently dry, no visible surface humidity, to prevent spread of colonies (eg. by drying for 15 min at 56 $^\circ\text{C}$ in an incubator).
- During the NF VALIDATION study, *Ralstonia mannitolytica* has shown typical colonies on RAPID' *Campylobacter* after 48h of incubation. This strain, rarely found in food matrices, gives negative confirmation. It gives atypical agglutination reaction with the *Campylobacter* Confirm Latex (**code 356-4297**):
 - Weak and atypical in appearance (mucoid / curd) rather than the normal particulate agglutination with poor background clearing, grainy, homogeneous and dense.
- If the colonies are not easily countable after 24h of incubation, re-incubate the plate until 48h.
- In the event of discordant results (positive on RAPID' *Campylobacter* agar, negative by the confirmatory method), the laboratory must implement sufficient resources to ensure the validity of the result.

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PERFORMANCE

The growth performance of the media are verified with the following reference strains:

MICRO-ORGANISMS	Aspect after 24 - 48h at 41.5 °C in microaerophilic atmosphere
<i>Campylobacter coli</i> ATCC 43478 (WDCM 00004)	Good growth Colonies brick-red
<i>Campylobacter jejuni</i> ATCC 29428 (WDCM 00156)	Good growth Colonies brick-red
<i>Escherichia coli</i> ATCC 8739 (WDCM 00012)	Total Inhibition
<i>Staphylococcus aureus</i> ATCC 25923 (WDCM 00034)	Total Inhibition

QUALITY CONTROL

All products manufactured and sold by Bio-Rad are under a quality assurance system, including raw materials and to the marketing of finished products. Each batch of finished product is subject to quality control and it is sold only if it complies with the acceptance criteria.

The documentation relating to the production and control of each batch is kept. The documentation relating to the production and control of each batch is kept.

KEY WORDS

RAPID' *Campylobacter* / *Campylobacter* / Meat products / Poultry products / Enumeration