## RAPID’Salmonella

<table>
<thead>
<tr>
<th>Ref#</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3563961</td>
<td>90 mm x 20 dishes</td>
</tr>
<tr>
<td>3563963</td>
<td>90 mm x 120 dishes</td>
</tr>
<tr>
<td>3564705</td>
<td>500g</td>
</tr>
</tbody>
</table>

### FIELD OF APPLICATION

RAPID’Salmonella is a chromogenic medium used for the detection of Salmonella spp. in the analysis of food products for human, animal consumption and in environmental samples.

### NF VALIDATION by AFNOR CERTIFICATION as per EN ISO 16140 protocol

RAPID’Salmonella has been certified NF VALIDATION as alternative to reference method NF EN ISO 6579, according to the ISO 16140 protocol, for the detection of Salmonella spp. in all food products for human and animal consumption (short protocol and double enrichment protocol) and in environmental samples (Short protocol only and primary production stage samples excluded).

### AOAC-RI VALIDATION

RAPID’Salmonella had been validated by the AOAC Research Institute under the Performance Tested Method Program for detection of Salmonella in raw chicken breast, eggs, cantaloupe and peanut butter (certificate # 050701). Typical colonies on RAPID’Salmonella are presumptive and should be confirmed by standard reference methods appropriate for the food type being tested.

### NORDVAL VALIDATION

RAPID’Salmonella (short protocol and double enrichment protocol but with RVS enrichment time 24 ± 2 h only) is NordVal validated as an alternative method to the reference standard EN ISO 6579, according to the ISO 16140 protocol, for the detection of Salmonella spp. in all food products for human, animal consumption and in environmental samples (Short protocol only and primary production stage samples excluded).

### STANDARD REFERENCES

- **USDA FSIS Microbiology Laboratory Guidebook**, Chapter 4.05 Isolation and Identification of Salmonella from Meat, Poultry and Egg Products. Online at [http://www.fsis.usda.gov/shared/PDF/MLG_4_0_5.pdf](http://www.fsis.usda.gov/shared/PDF/MLG_4_0_5.pdf)

### PRINCIPLE

RAPID’Salmonella agar allows the presumptive identification of Salmonella spp., by detecting C8-esterase activity. Simultaneous screening of β-glucosidase activity permits the differentiation of salmonella colonies from those of other enterobacteria.

After incubation, salmonella appear as readily identifiable typical magenta colonies whereas non-salmonella grow as blue or non colored colonies.

RAPID’Salmonella agar permits detection of motile and non-motile salmonella, as well as lactose-positive Salmonella, Salmonella Typhi and Salmonella Paratyphi.
STORAGE
- Pre-poured: at +2° to 8°C in a dark place.
- Dehydrated: at +2° to 8°C, in carefully sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritive Mix</td>
<td>14.5 g</td>
</tr>
<tr>
<td>Selective Agents</td>
<td>14 g</td>
</tr>
<tr>
<td>Chromogenic Mix</td>
<td>2.3 g</td>
</tr>
<tr>
<td>Agar</td>
<td>12.7 g</td>
</tr>
<tr>
<td>Distilled water qsp</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

Final pH = 7.2 ± 0.2

OTHER PRODUCTS AND MATERIALS REQUIRED (Not supplied and non-exhaustive list)

- **Buffered Peptone Water**:
  - 6 bottles of 225 ml: 3554179
  - 500 g: 3564684
  - 5 bags of 2.3 L: 3555789
  - 2 bags of 5 L: 3555790

- Selective supplement: RAPID' Salmonella capsules
  - 100 x Quantity for 250 ml: 3564710
  - RAPID' Salmonella capsules 10X (3564709), 100 x Quantity for 2.5 liters
  - RAPID' Salmonella Supplement - (code 3564712), 1 x QSP 100 analyses

Materials
- Scales- 200g capacity, sensitivity of 0.1 g
- Sterile weighing bags
- Stomacher
- Hotplate
- Magnetic stirrer
- Sterile Petri dishes (Ø = 90mm)
- Sterile Pasteur pipettes or inoculating loops
- Water-bath
- Thermostatically-controlled incubator or incubation room, precise to ±1°C

PREPARATION OF DEHYDRATED MEDIUM
Always shake bottle before use

Dissolve 43.5 g of powder in 1 litre of distilled water and mix until a homogeneous suspension is obtained. Heat gently, agitating frequently, then brings to the boil for less than 1 minute. DO NOT PROLONG HEATING. DO NOT AUTOCLAVE.

Cool the medium to 50°C. Dispense in Petri dishes and leave to dry.

Reconstitution ratio: 43.5 g / liter
500g of powder makes 11.5 liters of medium.

PROTOCOLS
STANDARD METHOD NF EN ISO 6579

Preparation of samples
According to standards for the product concerned.

Enrichment
According to standards for the product concerned.

Inoculation and incubation
Streak 10 µL of the enrichment broth at end of incubation onto RAPID' Salmonella and X.L.D. agar (3541751 and 3569124).
Incubate at 37 ± 1°C for 24 ± 3 h for X.L.D. agar and 24 ± 2 h for RAPID' Salmonella.
ALTERNATIVE METHODS

- **RAPID’Salmonella** - Short protocol

**Preparation of samples**
Dilute $\eta \times g$ or $\eta \times mL$ of sample in 9 x $\eta$ mL of Buffered Peptone Water

*Note 1: Either the whole capsule or its contents only can be added before the stomacher phase. In order to make handling easier, we recommend opening the capsule and pouring out the contents (see PRECAUTIONS FOR USE).*

**Note 2: In the context of NF VALIDATION mark, no samples of over 25 g were tested.**

**Sample preparation with addition of the selective supplement as a concentrated solution to the enrichment broth**

The capsule contents and RAPID’Salmonella Supplement boxes (3564712) can be diluted in Buffered Peptone Water or Sterile Distilled Water for incorporation in liquid form.

- Dilute $\eta \times g$ or $\eta \times mL$ of the sample in 9 x $\eta$ mL of Buffered Peptone Water. Homogenise in a Stomacher blender.

- Where RAPID’S’Salmonella QSP 250 ml capsules (3564710) are used: Open n capsules and pour the contents directly into n x 10 mL Buffered Peptone Water to obtain a concentrated supplement solution. Add $\eta \times 0.4$ ml of the concentrated supplement solution to the sample to be analysed. Homogenise by agitating vigorously.

- Where RAPID’S’Salmonella QSP 2.5 Liter capsules (3564709) are used: Open n capsules and pour their contents directly into an empty recipient. Fill with n x 10 mL Buffered Peptone Water or n x 10 mL of Sterile Distilled Water. Homogenise by agitating vigorously to obtain a red concentrated solution. Add $\eta \times 0.04$ ml of the concentrated supplement solution to the sample diluted in the Buffered Peptone Water. Homogenise by agitating vigorously.

- Where RAPID’S’Salmonella Supplement (3564712), 1 x QSP 100 analyses are used: Open the box and fill with 100 ml of Buffered Peptone Water or Sterile Distilled Water. Homogenise by agitating vigorously to obtain a red concentrated solution. Add $\eta \times 0.04$ ml of the concentrated supplement solution to the sample diluted in the Buffered Peptone Water. Homogenise by agitating vigorously.

**Note 1:** The concentrated solution, once reconstituted with Buffered Peptone Water or Sterile Distilled Water can be stored for 1 week at ambient temperature, or at +2-8°C.

**Note 2:** In the context of NF VALIDATION mark, no samples of over 25 grams were tested.

**Enrichment**
Incubate plates at $41.5 \pm 1^\circ C$ for $18 \pm 2$ h.

**Note:** After incubation, the enrichment broth may be stored in a refrigerator (2°-8°C) for 72 h, before inoculating RAPID’S’Salmonella.

**Inoculation and incubation**
Using a sterile loop, collect 10 $\mu$L of enrichment broth at end of incubation and inoculate RAPID’S’Salmonella plate by streaking for isolated colonies. Incubate at $37 \pm 1^\circ C$ for $24 \pm 2$ h.

**Reading**
Salmonella form magenta colonies on RAPID’S’Salmonella agar.

- RAPID’Salmonella – Double enrichment protocol
Preparation of samples
Dilute \( n \times g \) or \( n \times mL \) of sample in \( 9 \times n \) mL of Buffered Peptone Water

\textbf{e.g.: dilute 25 g or 25 mL of sample in 225 mL of Buffered Peptone Water broth to obtain a 1/10 dilution.}

Specific preparations (cocoa, acid foods, etc) are described in ISO 6579 standard.

Homogenise with an agitator like a Stomacher.

\textbf{Note: In the context of NF VALIDATION mark, no samples of over 25 grams were tested.}

\textbf{Enrichment}
Incubate at \( 37 \pm 1^\circ C \) for \( 18 \pm 2 \) h.

Transfer \( 0.5 \) mL of culture from the non-selective enrichment to \( 10 \) mL of pre-warmed RVS broth (3564324 and 3555773), Incubate at \( 41.5 \pm 1^\circ C \) for \( 6 \) -26 h.

In the case of the NF VALIDATION certified method, enrichment in RVS broth takes:

- \( 6 \) -26 h for seafood products, vegetables, dairy products and egg products
- \( 24 \pm 2 \) h for meat products and animal feedstuffs.

\textbf{Inoculation and incubation}
Using a sterile loop, collect 10 \( \mu L \) of enrichment broth at end of incubation and inoculate RAPID’\textit{Salmonella} plate by streaking for isolation. Incubate at \( 37 \pm 1^\circ C \) for \( 24 \pm 2 \) h.

\textbf{Reading}
\textit{Salmonella} form magenta colonies on RAPID’\textit{Salmonella} agar plate.

\textbf{CONFIRMATION OF POSITIVE RESULTS}
In the context of AOAC validation, confirm suspect isolated colonies according to classic confirmation test procedure described in the standard reference method.

In the context of the NF VALIDATION mark, all samples identified as positive must be confirmed in one of the following ways:

\begin{itemize}
  \item Using the conventional tests described in the standardized methods by CEN or ISO (including the purification step)
  \item Using nucleic probes as described in ISO 7218 standard (eg. iQ-Check\textsuperscript{TM} \textit{Salmonella} II, 3578123) using isolated colonies (with or without purification step).
  \item Evaluation of oxidase activity (oxidase test, 3553834), followed by omnivalent Omni-O test (A60) (3560781) using 1 to 3 isolated suspect colonies. If reaction is positive to the Omni-O test, proceed with an ONPG biochemical test (3553822).
  \textit{Salmonella} are negative to oxidase test, positive to Omni-O test (A60) and negative to ONPG test, with the exception of lactose-positive \textit{Salmonella} which are ONPG+.
  \item Performing a latex agglutination test: 
  \textit{Salmonella} latex (3556710) test on an isolated colony. \textit{Salmonella} of groups B to E and G are positive to the latex test, or performing a \textit{Salmonella} Confirm Latex test, using an isolated colony (3556711). Oxoid \textit{Salmonella} Latex test was also validated.
  \item Use of any other NF VALIDATION certified method based on a different principle from that of RAPID’\textit{Salmonella}.
  The validated protocol of the second method must be respected in its entirety, i.e. all steps preceding the intermediary stage used as departure point for confirmation must be common to both methods.
\end{itemize}

\textit{In the event of discordant results (presumptive positive with RAPID’\textit{Salmonella}, negative with confirmation method and especially by the Latex test) the laboratory must follow the necessary steps to ensure validity of the result obtained. .}

\textbf{LIMITATION OF USE}
\begin{itemize}
  \item ONPG confirmation test excludes confirmation of lactose positive \textit{Salmonella}.
  \item Although the most prevalent \textit{Salmonella} strains can be detected by \textit{Salmonella} Confirm Latex kit, it must be noted that during the NF VALIDATION extension of RAPID’\textit{Salmonella}, \textit{Salmonella} Confirm Latex kit not allowed the detection of 41 of the 150 tested strains.
  \item Some strains of \textit{Salmonella} (a few are part of Dublin serovar and \textit{S. bongori} species) can show a weak magenta color due to a low esterase activity.
\end{itemize}
PRECAUTIONS

- Respect of Good Laboratory Practice (eg. EN ISO 7218). Appropriate protection, such as gloves and lab coat, should be worn when working with potentially infectious live bacteria such as *Salmonella*.
- Media that have come in contact with food samples should be considered contaminated and should be disposed of in accordance with local rules and regulations.
- If the whole capsule is added to the Buffered Peptone Water, sterile tweezers must be used to add the capsule to the bag. We recommend that you check that the capsule actually opened during the stomaching stage.
- If the capsule contents are handled with the fingers, it cannot be added to the enrichment broth due to the risk of contamination.
- The RAPID Salmonella capsule and RAPID Salmonella Supplement (3564712) contain selective agents and an excipient. Selective agents dissolve very well. The excipient however, remains in suspension and may create a deposit when the contents of the capsule are diluted in a small quantity of Buffered Peptone Water or Sterile Distilled Water. Always shake well therefore before using the concentrated solution.
- End of NF VALIDATION: please see the certificate BRD: 07/11–12/05. This certificate is available from Bio-Rad representative or AFNOR Certification.

(See SDS for Product Safety Information, [www.bio-rad.com](http://www.bio-rad.com))

REMARKS

As with all chromogenic media, it is essential to carry out streak inoculation so as to obtain correctly isolated colonies.

TECHNICAL SUPPORT IN THE UNITED STATES

In the United States, for technical assistance please call (800) 4BIORAD. Select option 2 for technical support and option 2 again for the food science division. To place an order, please call (800) 4BIORAD and press option 1 for customer care.

QUALITY CONTROL

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product.

Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria. Documentation relative to the production and control of each batch is kept on file.

QUALITY AND PERFORMANCE OF THE TEST

See quality certificate available on [www.bio-rad.com/certificate](http://www.bio-rad.com/certificate) (Catalog# / ref# and Lot# number are required)

KEY WORDS

RAPID Salmonella / Salmonella / Food products / Detection / Enumeration / Chromogenic / Medium.

BIBLIOGRAPHY


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