

Read instructions carefully before starting test

Reveal[®] 2.0

for Salmonella



NEO 35/01 – 10/11

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

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VALIDATIONS

Protocol number	Protocol	Matrices	Validation Status
1	Revive 37 ± 1°C 5 ± 1 hour 2xRV 41.5±1°C for 18 ± 2 hours Reveal test procedure	All processed food, except vegetables, low-moisture products, cheeses, milk and egg products	NF Validation
2	1x RV 41.5±1°C for 22 ± 2 hours Reveal test procedure	Poultry rinses	* Please refer to the AOAC kit insert for validated method for pet food
		All non-processed food except vegetables, milk, cheeses and egg products	NF Validation
3	Revive 37 ± 1°C 5 ± 1 hour 2xRV 41.5±1°C for 18 ± 2 hours M-Broth 37 ± 1°C for 7 ± 1 hours. Reveal test procedure	Dried pet food, highly viscous products, highly pigmented material	* Please refer to the AOAC kit insert for validated method for pet food
4	Revive 37 ± 1°C for 24 ± 2 hours. 2xRV 41.5±1°C for 24 ± 2 hours Reveal test procedure	Low moisture food products: milk powder, flours, chocolate, cocoa powder, peanut butter	* Please refer to the AOAC kit insert for validated method for powdered milk, peanut butter
5	BPW 37 ± 1°C for 18 ± 2 hours. RV 41.5 ± 1°C for 24 ± 2 hours. Reveal Test procedure	Milk, cheeses	* Please refer to the AOAC kit insert for validated method for powdered milk, peanut butter
		Egg products	NF Validation

INTENDED USE

The Reveal 2.0 for *Salmonella* test system provides for the rapid recovery of *Salmonella* in food, animal feed, and environmental samples allowing detection and presumptive identification of the test organism, generally within 24 hours.

In an AOAC Research Institute Performance Tested MethodSM study, the Reveal 2.0 for *Salmonella* test system was found to be an effective procedure for detection of *Salmonella enterica* in the following sample types: Processed food, poultry rinses, dried pet food, highly viscous products, milk powder, egg powder, flours, chocolate, cocoa powder, peanut butter, milk, cheeses, and egg products.

The Reveal 2.0 for *Salmonella* test kit has been certified by NF VALIDATION as an alternative to the reference method NF EN ISO 6579-1, according to the ISO 16140-2 protocol, for the detection of *Salmonella* spp. in meat products, egg products, seafood products, multi-component foods, or meal components product. For more information about the end of validity of the NF VALIDATION certification, please refer to the certificate NEO 35/01 – 10/11 available on the website: <http://nf-validation.afnor.org/en> and/or on request from NEOGEN. In the context of the NF VALIDATION, test portions weighing more than 25 g have not been tested.

The Reveal 2.0 test detects *Salmonella enterica* serovars of somatic groups A-E (with the exception of *Salmonella* Paratyphi A). This includes the most common serovars from both food and non-food sources^{1,2} however serovars that are not within somatic groups A-E will not be detected.

ASSAY PRINCIPLES

The system utilizes REVIVE medium which provides *Salmonella* with readily available nutrients and other factors required for its recovery from a stressed or injured condition. After a brief enrichment in REVIVE, selective enrichment in Rappaport-Vassiliadis (RV) then favors *Salmonella* growth to levels detectable by the Reveal test device which is described below.

For certain matrices, the initial non-selective enrichment step may be bypassed and the sample is introduced directly into the RV medium.

An available third enrichment option enables the Reveal test device to detect *Salmonella* spp. in matrices that are more difficult due to viscosity, pigment, or containing an extremely high level of nonviable cells. M-Broth was selected as this post enrichment as it is established in several standard procedures recommended in enzyme assays used in the food industry.

For optimal results with milk, cheeses, and egg products, samples are tested following enrichment in buffered peptone water and Rappaport-Vassiliadis broth in accordance with the ISO 6579 reference procedure for *Salmonella* spp.³

A portion (200 µL) of the enrichment culture is placed into the sample cup. The test device is placed into the sample and allowed to develop at ambient temperature for 15 minutes. The sample is wicked through a reagent zone which contains specific antibodies (anti-*Salmonella*) conjugated to colloidal gold particles. If antigens are present in the sample, they will bind to the gold conjugated antibodies. This antigen-antibody complex then leaves the reagent zone and travels through the nitrocellulose membrane which contains a zone of anti-*Salmonella* antibodies. The immune complex with gold conjugate is captured and aggregates in this zone, thus displaying a visible line. The remainder of the sample continues to migrate to the end of the membrane where it is deposited into a waste reservoir.

The reagent zone also contains gold conjugate of a proprietary antigen which is eluted by the sample regardless of the presence of *Salmonella* antigen. The gold-conjugated control indicator migrates through the membrane to the negative control capture zone (antibody to the proprietary antigen), where it is captured and aggregated to form a visible line. Regardless of the presence or absence of the *Salmonella* antigen, the control line will form in the control zone, ensuring the test is working properly.

INTENDED USER

The Reveal 2.0 for *Salmonella* test system is designed for use by personnel familiar with the appropriate aseptic techniques for the isolation and identification of *Salmonella*. Training, which is available through NEOGEN, is recommended for those without a basic knowledge of microbiology. Comply with good laboratory practices (refer to EN ISO 7218 standard).

MATERIALS PROVIDED

Reveal Test Kit

- 20 Reveal 2.0 for *Salmonella* test devices
- 20 disposable transfer pipettes
- 20 reaction cups

Revive Media Kit for Protocols 1, 3 & 4

- 20 pouches of REVIVE medium
- 20 Stomacher-type bags
- Graduated cup

Secondary Selective Enrichment Kit for Protocols 1, 3 & 4

- 20 pouches 2x concentrated Rappaport-Vassiliadis (2xRV)
- 20 Stomacher bags
- Graduated cup

Direct Selective Enrichment Kit for Protocol 2

- 20 pouches 1x Rappaport-Vassiliadis (1xRV)
- 20 Stomacher bags
- Graduated cup

M-Broth Post-Enrichment Kit for Protocol 3

- 20 bottles M-Broth

MATERIALS REQUIRED BUT NOT PROVIDED

- Scale capable of weighing a minimum of 25 g
- Timer
- Sample cup rack
- Sterile (deionized or distilled) water
- Incubator capable of maintaining $41.5\pm 1^{\circ}\text{C}$
- Incubator capable of maintaining $37\pm 1^{\circ}\text{C}$

Optional Materials

- Reveal AccuScan® III Reader
- Stomacher 400 machine or equivalent

STORAGE

Store Reveal devices between 15–30°C when not in use. Store dry media at room temperature (15–30°C).

I. SAMPLE PREPARATION

Protocol 1: (Revive/RV) – All processed food, except low-moisture products, cheeses, milk and egg products

1. Transfer contents of 1 pouch of unitized REVIVE (9705) or 7.2 g of bulk REVIVE (9708) into a Stomacher-type bag. Using the graduated cup, add 200 mL of sterile-purified water pre-warmed to $41.5 \pm 1^\circ\text{C}$. Grasp the bag tightly 2–3 inches from the top and mix vigorously until dissolved.
2. Place 25 g of food sample (sample must be at room temperature) or environmental sponge sample into the Stomacher-type bag containing the REVIVE medium. Grasp bag tightly at top and knead sample until mixed. Shake bag vigorously using a side-to-side motion to ensure complete mixing. **ALTERNATIVE:** Place bag in Stomacher apparatus and mix for 30 seconds at normal speed.
3. Loosely close the bag and place in suitable rack or support. Incubate $37 \pm 1^\circ\text{C}$ for 5 ± 1 hours.
4. Reconstitute 2xRV in a Stomacher bag by adding 1 pouch of unitized 2xRV concentrated (9715) or 10.6 g of bulk RV (9716). Using the cup provided, add 200 mL of sterile-purified water pre-warmed to $37 \pm 1^\circ\text{C}$ to the bag. Mix vigorously until dissolved. Hold prepared 2xRV at $41.5 \pm 1^\circ\text{C}$ until use.
5. Remove the REVIVE sample bag from the $37 \pm 1^\circ\text{C}$ incubator and place in a suitable rack or support.
6. Add the 200 mL of selective 2xRV enrichment pre-warmed to $41.5 \pm 1^\circ\text{C}$ to the entire REVIVE culture (200 mL) in the sample bag. Grasp tightly 2–3 inches from top and mix gently using a side-to-side motion.
7. Loosely close bag and place in a suitable rack or support. Incubate at $41.5 \pm 1^\circ\text{C}$ for 18 ± 2 hours.
8. Proceed according to Section II – Reveal Test Procedure.

NOTE: Enrichment cultures may be stored at $2\text{--}8^\circ\text{C}$ for up to 72 hours prior to testing with the Reveal device.

Protocol 2: (Direct RV) – Poultry rinses (excluded from NF VALIDATION scope) and all non-processed food except milk, cheeses, and egg products.

1. Transfer contents of 1 pouch of unitized 1xRV (9729) or 5.3 g of bulk RV (9716) into a Stomacher-type bag. Using the graduated cup, add 200 mL of sterile-purified water pre-warmed to $41.5 \pm 1^\circ\text{C}$. Grasp the bag tightly 2–3 inches from the top and mix vigorously until dissolved.
2. Place 25 g of sample (sample must be at room temperature) into the Stomacher-type bag containing the 1xRV medium. Grasp bag tightly at top and knead sample until mixed. Shake bag vigorously using a side-to-side motion to ensure complete mixing. **ALTERNATIVE:** Place bag in Stomacher apparatus and mix for 30 seconds at normal speed.
3. Loosely close the bag and place in suitable rack or support. Incubate $41.5 \pm 1^\circ\text{C}$ for 22 ± 2 hours.
4. Proceed according to Section II – Reveal test procedure.

NOTE: Enrichment cultures may be stored at $2\text{--}8^\circ\text{C}$ for up to 72 hours prior to testing with the Reveal device.

Protocol 3: (Revive/RV - M-Broth) – Dried pet food, highly viscous products, highly pigmented material (excluded from NF VALIDATION scope).

1. Transfer contents of one pouch of unitized REVIVE (9705) or 7.2 g of bulk REVIVE (9708) into a Stomacher-type bag. Using the graduated cup, add 200 mL of sterile-purified water pre-warmed to $41.5 \pm 1^\circ\text{C}$. Grasp the bag tightly 2–3 inches from the top and mix vigorously until dissolved.
2. Place 25 g of sample (sample must be at room temperature) into the Stomacher-type bag containing the REVIVE medium. Grasp bag tightly at top and knead sample until mixed. Shake bag vigorously using a side-to-side motion to ensure complete mixing. **ALTERNATIVE:** Place bag in Stomacher apparatus and mix for 30 seconds at normal speed.
3. Loosely close the bag and place in suitable rack or support. Incubate at $37 \pm 1^\circ\text{C}$ for 5 ± 1 hours.
4. Reconstitute 2xRV in a stomacher bag by adding 1 pouch of unitized 2xRV concentrated (9715) or 10.6 g of bulk RV (9716). Using the cup provided, add 200 mL of sterile-purified water pre-warmed to $37 \pm 1^\circ\text{C}$ to the bag. Mix vigorously until dissolved. Hold prepared 2xRV at $41.5 \pm 1^\circ\text{C}$ until use.
5. Remove the REVIVE sample bag from the $37 \pm 1^\circ\text{C}$ incubator and place in a suitable rack or support.
6. Add the 200 mL of selective 2xRV enrichment pre-warmed to $41.5 \pm 1^\circ\text{C}$ to the entire REVIVE culture (200 mL) in the sample bag. Grasp tightly 2–3 inches from top and mix gently using a side-to-side motion.
7. Loosely close bag and place in a suitable rack or support. Incubate at $41.5 \pm 1^\circ\text{C}$ for 18 ± 2 hours.
8. Rehydrate 1 bottle of M-Broth (9722) by adding 10 mL of sterile-purified water pre-warmed to $37 \pm 1^\circ\text{C}$. Cap tightly and shake to dissolve medium.
9. Remove 1 mL aliquot from the REVIVE/RV sample and transfer into the bottle of rehydrated M-Broth.
10. Incubate at $37 \pm 1^\circ\text{C}$ for 7 ± 1 hours.
11. Proceed according to Section II – Reveal test procedure.

NOTE: Enrichment cultures may be stored at 2–8°C for up to 72 hours prior to testing with the Reveal device.

Protocol 4: (Revive/RV) – Low moisture food products: milk powder, flours, chocolate, cocoa powder, peanut butter (excluded from NF VALIDATION scope).

1. Transfer contents of 1 pouch of unitized REVIVE (9705) or 7.2 g of bulk REVIVE (9708) into a Stomacher-type bag. Using the graduated cup, add 200 mL of sterile-purified water pre-warmed to $41.5 \pm 1^\circ\text{C}$. Grasp the bag tightly 2–3 inches from the top and mix vigorously until dissolved.
2. Place 25 g of food sample (sample must be at room temperature) or environmental sponge sample into the Stomacher-type bag containing the REVIVE medium. Grasp bag tightly at top and knead sample until mixed. Shake bag vigorously using a side-to-side motion to ensure complete mixing. **ALTERNATIVE:** Place bag in Stomacher apparatus and mix for 30 seconds at normal speed.
3. Loosely close the bag and place in suitable rack or support. Incubate $37 \pm 1^\circ\text{C}$ for 24 ± 2 hours.
4. Reconstitute 2xRV in a Stomacher bag by adding 1 pouch of unitized 2xRV concentrated (9715) or 10.6 g of bulk RV (9716). Using the cup provided, add 200 mL of sterile-purified water pre-warmed to $37 \pm 1^\circ\text{C}$ to the bag. Mix vigorously until dissolved. Hold prepared 2xRV at $41.5 \pm 1^\circ\text{C}$ until use.
5. Remove the REVIVE sample bag from the $37 \pm 1^\circ\text{C}$ incubator and place in a suitable rack or support.
6. Add the 200 mL of selective 2xRV enrichment pre-warmed to $41.5 \pm 1^\circ\text{C}$ to the entire

REVIVE culture (200 mL) in the sample bag. Grasp tightly 2–3 inches from top and mix gently using a side-to-side motion.

7. Loosely close bag and place in a suitable rack or support. Incubate at $41.5 \pm 1^\circ\text{C}$ for 24 ± 2 hours.
8. Proceed according to Section II – Reveal test procedure.

NOTE: Enrichment cultures may be stored at 2–8°C for up to 72 hours prior to testing with the Reveal device.

Protocol 5 (BPW - RV) – Milk, cheeses (excluded from NF VALIDATION Scope) and egg products

1. Homogenize 25 g sample in 225 mL Buffered Peptone Water (BPW). Incubate at $37 \pm 1^\circ\text{C}$ for 18 ± 2 hours.
2. Remove 0.1 mL aliquot of BPW culture and add to 10 mL RV broth. Incubate at $41.5 \pm 1^\circ\text{C}$ for 24 ± 2 hours.
3. Mix the enrichment and transfer 200 μL in a Reveal Sample cup (ref 9706).
4. Proceed according to Section II – Reveal test procedure.

NOTE: Enrichment cultures may be stored at 2–8°C for up to 72 hours prior to testing with the Reveal device.

II. REVEAL TEST PROCEDURE

1. Remove enriched sample from designated incubator. Mix sample well and transfer 200 μL or 8 drops to the Reveal sample cup.
2. Remove the required number of Reveal for *Salmonella* test devices from container.
3. Place Reveal device with sample arrows facing down into sample cup containing sample and incubate at ambient temperature for 15 minutes.
4. Observe and record the test results immediately (within 60 seconds) after 15 minutes incubation.

III. INTERPRETATION OF RESULTS

Visual Interpretation

- Line in both control and test zone in 15 minutes is considered positive.
- Line in control zone only at 15 minutes is considered negative.
- If no line appears in the control zone, the test is considered invalid and the sample should be retested with another device.
- Observations after 20 minutes may be inaccurate due to over-development of device.

NOTE: The Reveal device forms a distinct line in the test region when *Salmonella* are present; the intensity of the line may be variable based on serovar and/or concentration. If a distinct visible line forms, regardless of intensity, the sample should be considered positive. Migration of any blue pigment from the RV enrichment to the device does not impact results.

IV. CONFIRMATION OF POSITIVE RESULTS

In the context of NF VALIDATION, all samples identified as positive by the Reveal 2.0 for *Salmonella* test must be confirmed by one of the following tests:

- (1) Using the conventional tests described in the methods standardized by CEN or ISO from isolated colonies (including the purification step) from the last enrichment broth.
- (2) Streaking onto XLD or an esterase chromogenic agar plate followed by the tests of the ISO 6579, or a latex test performed directly on isolated colonies.

In the event of discordant results (presumptive positive with the alternative method, non-confirmed by one of the means described above, and in particular the latex test), the laboratory must follow the necessary steps to ensure the validity of the result obtained.

V. REFERENCES

¹ Galanis, E. et al. (2006) Emerg. Infect. Dis. 12, 381-388

² www.who.int/salmsurv

³ ISO (2002) ISO 6579:2002 (E) - Horizontal method for the detection of *Salmonella* spp.

CUSTOMER SERVICE

NEOGEN Customer Assistance and Technical Service can be reached by using the contact information on the back of this booklet. Training on this product, and all NEOGEN test kits, is available.

SDS INFORMATION AVAILABLE

Safety data sheets (SDS) are available for this test kit, and all of NEOGEN's Food Safety test kits, at NEOGEN.com, or by calling NEOGEN at 800.234.5333 or 517.372.9200.

TERMS AND CONDITIONS

For NEOGEN's full terms and conditions, please visit neogen.com/terms-and-conditions/

WARRANTY

NEOGEN Corporation makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, NEOGEN will provide a replacement product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. NEOGEN shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

PRODUCTS AVAILABLE

- 9706 **Reveal 2.0 for *Salmonella*** – a one-step assay for detection of *Salmonella*. Kit contains 20 devices.
- 9705 **REVIVE for *Salmonella*** – patented resuscitation medium to speed up enrichment cultivation for *Salmonella*. Contains 20 pouches and 20 Stomacher bags for 20 tests.
- 9708 **Bulk REVIVE for *Salmonella*** – 500 g
- 9729 **1x RV Selective Media** – Rappaport-Vassiliadis broth 1x. Media kit contains prefilled pouches for 20 tests and 20 Stomacher-type bags.
- 9715 **2x RV Selective Media** – Rappaport-Vassiliadis broth 2x concentrate. Media kit contains prefilled pouches for 20 tests and 20 Stomacher-type bags. To be used in combination with REVIVE.
- 9716 **Bulk RV Media** – 500 g
- 9724 ***Salmonella* Confirmation Kit** – contains *Salmonella* magnetic beads, buffer packets, and Rambach Chromogenic agar and supplement for 20 tests.
- 9724B **Bulk *Salmonella* Confirmation Kit** – contains *Salmonella* magnetic beads for 50 tests.
- 7649 **Rambach Agar for *Salmonella*** – chromogenic agar for *Salmonella* (4 x 250 mL).
- 9427 **Electronic digital scale**
- 9735 **Incubator**
- 9426 **Timer (3 channel)**
- 9415 **Pipettes** – sterile 10 mL serological (500).
- 9722 **M-Broth** – media kit contains prefilled bottles for 20 tests.
- 9475 **Rack for Reveal Sample Cups**
- 9802 **Reveal 2.0 One Step System** – contains devices, 1xRV selective medium and sampling bags for 20 tests.
- 9803 **Reveal 2.0 Complete System with RV** – contains devices, REVIVE, 2xRV selective medium and sampling bags for 20 tests.
- 9804 **Reveal 2.0 Environment System** – contains devices, Revive, 2xRV, sampling bags and environmental sample kits for 20 tests.
- 9805 **Reveal 2.0 Complete System with RV and M-Broth** – contains devices, Revive, 2xRV selective medium, M-Broth and sampling bags for 20 kits.



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