

U.S. English

Product Number:
9706

Reveal[®] 2.0

for Salmonella



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Intended Use

The Reveal[®] 2.0 for *Salmonella* test system provides for 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 Method[™] 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: powdered milk, ice cream, raw ground beef, raw ground chicken, raw ground pork, raw ground sausage, raw ground turkey, cooked chicken, hot dogs, ready-to-eat meal product, chicken carcass rinse, beef skin, pork skin, picked crab meat, raw shrimp, cantaloupe, fresh spinach, peanut butter, liquid eggs, frozen whole eggs, dry pet food, soybean meal, poultry feed, sprout irrigation water, and sponge samples from stainless steel surface

Antibodies used in the Reveal 2.0 for *Salmonella* test are reactive with *Salmonella enterica* serovars of somatic groups A–E. This includes the most common serovars from both food and non-food sources.^{1,2} 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.

For certain matrices, the initial nonselective enrichment step may be bypassed and the sample introduced directly into the RV medium.

Another 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 for post enrichment as it is established in several standard procedures recommended in enzyme assays used in the food industry.

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 that contains specific anti-*Salmonella* antibodies 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.

Materials Provided

1. 20 Reveal 2.0 for *Salmonella* test devices
2. 20 disposable transfer pipettes
3. 20 reaction cups

Test Systems Required but Not Provided

Note: The Reveal 2.0 for *Salmonella* device requires the use of certain enrichment media and supplies. These can be purchased as part of a test system or individually.

1. Reveal 2.0 one-step system (contains devices, 1x RV selective medium, and sampling bags for 20 tests) (Neogen item 9802)
2. Reveal 2.0 complete system with RV (contains devices, Revive, 2x RV selective medium, and sampling bags for 20 tests) (Neogen item 9803)
3. Reveal 2.0 environmental system (contains devices, Revive, 2x RV, sampling bags, and environmental sample kits for 20 tests) (Neogen item 9804)
4. Reveal 2.0 complete system with RV and M-broth (contains devices, Revive, 2x RV selective medium, M-broth, and sampling bags for 20 tests) (Neogen item 9805)

Media Required but Not Provided (Depending on Sample)

Note: Media can be purchased individually or as part of a test system.

1. 20 foil pouches of Revive medium (Neogen item 9705)
2. 20 foil pouches 2x concentrated Rappaport-Vassiliadis (2x RV) (Neogen item 9715)
3. 20 foil pouches 1x Rappaport-Vassiliadis (1x RV) (Neogen item 9729)
4. 20 bottles M-Broth (Neogen item 9722)
5. Lactose Broth (Neogen item 7141)

Materials Required but Not Provided

1. Scale capable of weighing a minimum of 25 g (Neogen item 9427)
2. Timer (Neogen items 9452, 9426)
3. Sample cup rack (Neogen item 9475)
4. Sterile deionized or distilled water
5. Incubator capable of maintaining $42 \pm 1^\circ\text{C}$ (Neogen item 9735)
6. Incubator capable of maintaining $36 \pm 1^\circ\text{C}$ (Neogen item 9735)
7. Graduated cup
8. Stomacher-type bags

Optional Materials

1. AccuScan® reader
2. Stomacher 400 machine or equivalent

Storage

Store Reveal devices and dry media at room temperature (15–30°C) when not in use. Store M-broth between 2–8°C.

Testing of Different Commodities

Please contact a Neogen representative for an updated list of the commodities for which the test has been validated, or for validation studies for specific commodities.

Precautions

1. Do not use expired media or test devices.
2. Use rehydrated media within 6 hours of preparation.
3. Use of incubation times and temperatures other than those specified may lead to erroneous results.
4. Sample bags must be closed loosely to allow air exchange during incubation, which is vital for sufficient and detectable growth.
5. Sterile water should be warmed to 42°C when rehydrating Revive and 36 ± 1°C when rehydrating 2x RV and 1x RV.
6. Test enriched cultures within 6 hours post-incubation.
7. Good microbiological laboratory practices should be used.

Sample Preparation and Enrichment

Sample Enrichment 1 (Revive/RV) — For Most Foods and Environmental Samples

Validated for³ cantaloupe, ice cream, ready-to-eat meat products, and stainless steel surfaces. For use with Neogen items 9803 and 9804.

1. Transfer contents of 1 foil pouch of unitized Revive (Neogen item 9705) or 7.2 g of bulk Revive (Neogen item 9708) into a Stomacher-type bag. Using the graduated cup, add 200 mL of sterile-purified water prewarmed to 42°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 36 ± 1°C for 4 hours.
4. Reconstitute 2x RV in a Stomacher-type bag by adding 1 foil pouch of unitized 2x RV concentrated (Neogen item 9715) or 10.6 g of bulk RV (Neogen item 9716). Using the cup provided, add 200 mL of sterile purified water prewarmed to 36 ± 1°C to the bag. Mix vigorously until dissolved. Hold prepared 2x RV at 42°C until use.
5. Remove the Revive sample bag from the 36 ± 1°C incubator and place in a suitable rack or support.
6. Add the 200 mL of selective 2x RV enrichment prewarmed to 42°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 42 ± 1°C for 16–24 hours.

Sample Enrichment 2A (Direct RV) — For Select Foods Including Raw Ground Beef, Raw Ground Poultry, Raw Seafood, and Poultry Rinses

Validated for³ chicken carcass rinse, raw ground turkey, raw ground beef, and raw shrimp. For use with Neogen item 9802.

1. Transfer contents of 1 foil pouch of unitized 1x RV (Neogen item 9729) or 5.3 g of bulk RV (Neogen item 9716) into a Stomacher-type bag. Using the graduated cup, add 200 mL of sterile purified water prewarmed to 42°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) or 30 mL poultry carcass rinse into the Stomacher-type bag containing the 1x RV 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 42 ± 1°C for 20–24 hours.

Sample Enrichment 2B (Direct RV) — For Deli Meats and Other Ready-to-eat Meat Products

Validated for³ hot dogs. For use with Neogen item 9802.

1. Add 68.9 g of bulk RV (Neogen item 9716) into a large Stomacher-type bag.
2. Add 2.6 L of sterile-purified water prewarmed to 42°C. Grasp the bag tightly 2–3 inches from the top and mix vigorously until dissolved.
3. Place 325 g of sample (sample must be at room temperature) into the Stomacher-type bag containing the 1x RV medium. Grasp bag tightly at the 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.

4. Loosely close the bag and place in a suitable rack or support. Incubate at 42 ± 1°C for 20–24 hours.

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Sample Enrichment 2C (Direct RV)

Validated for³ sprout irrigation water. For use with Neogen item 9706.

1. Add 19.9 g bulk RV (Neogen item 9716) to a large Stomacher-type bag. Add 375 mL sterile-purified water prewarmed to 42°C. Grasp the bag tightly 2–3 inches from the top and mix vigorously until dissolved.
2. Add 375 mL sprout irrigation water to the Stomacher-type bag containing the 2x RV medium. Grasp bag tightly at the 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 a suitable rack or support. Incubate at 42 ± 1°C for 20–24 hours.

Sample Enrichment 3 (Revive/RV — M-Broth) — For Matrices That May Contain Residual Antigen, Highly Viscous, or Highly Pigmented Material

Validated for³ dry pet food. For use with Neogen item 9805.

1. Transfer contents of 1 foil pouch of unitized Revive (Neogen item 9705) or 7.2 g of bulk Revive (Neogen item 9708) into a Stomacher-type bag. Using the graduated cup, add 200 mL of sterile-purified water prewarmed to 42°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 36 ± 1°C for 4 hours.
4. Reconstitute 2x RV in a Stomacher bag by adding 1 foil pouch of unitized 2x RV concentrated (Neogen item 9715) or 10.6 g of bulk RV (Neogen item 9716). Using the cup provided, add 200 mL of sterile-purified water prewarmed to 36 ± 1°C to the bag. Mix vigorously until dissolved. Hold prepared 2x RV at 42°C until use.
5. Remove the Revive sample bag from the 36 ± 1°C incubator and place in a suitable rack or support.
6. Add the 200 mL of selective 2x RV enrichment prewarmed to 42°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 42 ± 1°C for 16–24 hours.
8. Rehydrate 1 bottle of M-broth (Neogen item 9722) by adding 10 mL of sterile-purified water prewarmed to 36 ± 1°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 36 ± 1°C for 6 hours.

Sample Enrichment 4 (RV — M-broth) — For Leafy Greens

Validated for³ fresh spinach. For use with Neogen item 9802 and M Broth.

1. Transfer contents of 1 foil pouch of unitized 1x RV (Neogen item 9729) or 5.3 g of bulk RV (Neogen item 9716) into a Stomacher-type bag. Using the graduated cup, add 200 mL of sterile-purified water prewarmed to 42°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 1x RV 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 42 ± 1°C for 20–24 hours.
4. Rehydrate 1 bottle of M-broth (Neogen item 9722) by adding 10 mL of sterile-purified water prewarmed to 36 ± 1°C. Cap tightly and shake to dissolve medium.
5. Remove 1 mL aliquot from the RV sample and transfer into the bottle of rehydrated M-broth.
6. Incubate at 36 ± 1°C for 6 hours.

Sample Enrichment 5 (Lactose — RV)

Validated for³ peanut butter. For use with Neogen item 9802 and Lactose Broth

1. Homogenize 25 g sample in 225 mL Lactose Broth. Incubate at 36 ± 1°C for 22–24 hours.
2. Remove 0.1 mL aliquot of Lactose Broth culture and add 10 mL RV broth. Incubate at 42 ± 1°C for 22–24 hours.

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.
 - a. After transferring the enriched sample to the sample cup, do not allow the sample to sit more than 10 minutes before placing the Reveal device into the sample cup (steps 2–3).
2. Remove the required number of Reveal 2.0 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.

Interpretation of Results

Visual Interpretation

1. Line in both control and test zone in 15 minutes is considered positive.
2. Line in control zone only at 15 minutes is considered negative.
3. If no line appears in the control zone, the test is considered invalid and the sample should be retested with another device.

Optional Electronic Interpretation

Place the Reveal test device into the AccuScan reader according to the instrument instructions and follow the onscreen prompts to interpret and record the device's test result.

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.

Confirmation

Neogen recommends that presumptively positive Reveal enrichment cultures be verified by plating a sample of the culture following methods described in BAM⁴ or MLG⁵.

Note: Testing a replicate subsample using BAM or MLG procedures may not yield the same result. Bacteria are not evenly distributed with a lot of material hence the subsample may not contain the organism at the onset.

Disposal

Dispose (autoclave, bleach, etc.) of Reveal test devices, pipettes, and media in accordance with all applicable local, state, and federal regulations.

Customer Service

Neogen Customer and Technical Services can be contacted through neogen.com and product training is available by request.

SDS Information Available

Safety data sheets are available for all test kits at neogen.com or by calling 800.234.5333 or 517.372.9200.

Terms and Conditions

Neogen's full terms and conditions are available online.

Warranty

Neogen 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 of the 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.

References

^[1,2]Galanis, E. et al. (2006) Emerg. Infec. Dis. 12, 381-388, www.who.int/salmsurv

^[3]In an AOAC Research Institute Performance Tested MethodSM study

^[4]<http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM070149>

^[5]http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp

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