Assurance® GDS *Salmonella* Tq

There are three validated methods that can be followed:

AOAC® Official Method of Analysis 2009.03
AOAC® Performance Tested Method 050602
Health Canada Method MFLP-36

Part No: 71008-100 (100 tests)
71008-576 (576 tests)
71008-576ATM (576 tests)

General Description

Assurance® GDS, genetic detection system, for *Salmonella* Tq is an automated nucleic acid amplification system for the detection of *Salmonella* in meats, poultry, poultry rinse, seafood, dairy products, spices, egg, pasta, chocolate and bakery products, peanut butter, fruits and vegetables, and environmental surfaces .

Kit Components

Each Assurance® GDS for *Salmonella* Tq kit contains the following:

- Amplification Tubes Tq
- Concentration Reagent
- Resuspension Buffer Tq
- Wash Solution

Each Assurance® GDS for *Salmonella* Tq 576ATM kit contains the following:

- Amplification Tubes Tq
- Concentration Reagent

The following are also necessary but sold separately:

- 61031-100 Wash Solution Kit
- 34724-100C Resuspension Buffer Tq

Equipment / Materials Required

Other necessary materials not provided include:

- Media per Appendix A
- Assurance® GDS Rotor-Gene®
- PickPen® device and PickPen® tips
- Vortex mixer
- Adhesive film strips
- Sample wells and sample wells base
- Resuspension plate
- Gel cooling block
- Stomacher / Masticator or equivalent

- 8-channel micropipette capable of dispensing 30 µL
- Repeat pipette
- Adjustable micropipettes
- Repeat pipette tips (0.5 mL and 10 mL)
- Filter barrier micropipette tips (50 µL and 1.0 mL)
- Incubator capable of maintaining 35 – 37 °C

Additional materials for the 576 kit include:

- Variable Spacing Multi-Channel Pipette
- Aluminum Cooling Block, 72 well
- 72-well rotor and locking ring

AOAC® Official Method of Analysis 2009.03

Approved matrices include: Raw Beef, Raw Pork, Ground Poultry, Poultry Rinse, Raw Fish, Raw Shrimp, Orange Juice, Raw Spinach, Raw Tomato, Cantaloupe, Peanut Butter, Spices, Liquid Milk, Nonfat Dry Milk, Ice Cream, Pasta, Eggs and Environmental Surfaces.

Sample Preparation

A. Test Portion Preparation & Enrichment

a. Add 25 g (mL) of sample to 225 mL of Buffered Peptone Water (BPW).

For **raw seafood**, add 25 g of sample to 225 mL BPW with novobiocin (Appendix A).

For **non-fat dry milk**, add 25 g of sample to 225 mL Brilliant Green Water (Appendix A).

For **spice** (cumin, curry powder and chili powder), add 25 g or samples to 225 mL Tryptic Soy Broth (TSB) (Appendix A). For preparations of initial suspension, follow instructions of BAM Chapter 5: *Salmonella*. Adjust, if needed, to pH 6.6 - 7.0.

For **environmental** monitoring, pre-moisten sterile dehydrated sponges with 10 mL D/E (Dey/Engley) Broth or Letheen Broth. Hydrate sterile swab by soaking in D/E or Letheen broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of BPW, respectively. If alternate test portion sizes are analyzed, proportionately adjust the volume of media to maintain 1:9 ratio.

For **poultry rinse**, follow USDA MLG Chapter 4 for sample size and enrichment media volume.

b. Homogenize or mix sample and incubate samples for 18 – 24 h at 35 – 37 °C. Incubate **non-fat dry milk** and **spice** samples for 20 – 24 h at 35 – 37 °C.

c. For **high microbial load**, **non-fat dry milk** and **spice** samples, transfer enriched samples to BHI for 2 – 4 h at 35 – 37 °C as described beginning with step **B** (**h)**.

Note: Contact us for recommended procedures for testing alternate sample sizes.

B. Sample Processing Protocol

Change gloves prior to handling reagents.

a. Vortex **Concentration Reagent**. Immediately transfer 20 µL to each of the required number of Assurance® GDS sample wells (1 well/sample) using a repeat pipette and 0.5 mL pipette tip. Cover sample wells with adhesive film strips.

b. For **low microbial load** and **raw seafood**, transfer 1.0 mL of **Wash Solution** to additional sample wells (1 well/sample) held in a sample well base using a repeat pipette and 10 mL pipette tip. Cover sample wells with adhesive film strips.

   For **high microbial load** and **non-fat dry milk samples**, dispense 0.5 mL of sterile Brain Heart Infusion (BHI) broth to sample wells (1 well /sample) in place of Wash Solution. Cover sample wells with adhesive film strips.

   For **spice** samples, dispense 0.5 mL of sterile Brain Heart Infusion (BHI) broth to sample wells (1 well /sample). Dispense 1.0 mL of Wash Solution to each of 2 additional sample wells (2 well/sample). Cover sample wells with adhesive film strips.

   For **raw ground poultry**, add 0.7 mL of Wash Solution to the sample wells containing Concentration Reagent and dispense 0.5 mL of sterile BHI to a separate set of sample wells. Cover all sample wells with adhesive film strips.

c. Transfer 45 µL of **Resuspension Buffer Tq** to the wells in the resuspension plate using a repeat pipette and a 0.5 mL pipette tip. Cover resuspension plate with adhesive film strips.

d. After incubation, carefully remove adhesive film from 1 strip of sample wells. Add 1.0 mL of incubated sample to each sample well containing Concentration Reagent.

   For **raw ground poultry**, transfer 0.3 mL of incubated sample to wells containing Concentration Reagent and Wash Solution.

   Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film strip prior to adding samples to a new strip. **Return samples to incubator.**

e. Place sealed sample wells on the vortex mixer and vortex at approximately 900 rpm for 10 – 20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.

f. Carefully remove and discard adhesive film strip from a strip of samples. Remove corresponding film strip from a strip of sample wells containing Wash Solution or BHI.

g. When analyzing **peanut butter** samples, load PickPen® tips onto PickPen® tool. Insert tips into the sample wells without extending the magnets. Stir 10 s to remove excess fat accumulation. Discard tips and continue with step (h).

h. For all samples load tips onto the PickPen® device, ensuring that the tips are firmly in place on the PickPen® tool. Extend the PickPen® magnets and insert tips into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Gently tap the PickPen® tips against the side of the sample wells to remove excess media droplets.

i. For **low microbial load** and **raw seafood** samples, transfer PickPen® tips to corresponding sample wells containing Wash Solution and gently swirl for 10 s (do not release particles into solution). Transfer PickPen® tips to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen® magnets and tap tips gently to release particles into the Resuspension Buffer Tq. Cover resuspension plate with adhesive film strips and continue with step (l).

j. For **high microbial load**, **non-fat dry milk** and **raw ground poultry** samples, transfer PickPen® tips to corresponding sample wells containing BHI. With tips submerged, retract the PickPen® magnets and tap gently to release particles into the BHI. Cover each BHI strip with a new adhesive film strip prior to adding samples to a new strip. Incubate sample wells containing BHI and particles for 2 – 4 h at 35 – 37 °C. For **raw ground poultry**, incubate sample wells with BHI for a minimum of 3 h.

   Following incubation, transfer the particles from the BHI sample wells to the corresponding row of the prepared resuspension plate using the PickPen®, as indicated in steps B(f) – B(j). With tips submerged, retract the PickPen® magnets and tap tips gently to release particles into the Resuspension Buffer Tq. Cover the resuspension plate with adhesive film and continue with step (l).

k. For **spice** samples, transfer PickPen® tips to 1st set of sample wells containing Wash Solution and gently swirl for 10 s (do not release particles into solution). Next, transfer PickPen® tips to corresponding sample wells containing BHI. With tips submerged, retract the PickPen® magnets and tap gently to release particles into the BHI. Cover each strip with a new adhesive film strip prior to adding samples to a new strip. Incubate sample wells containing BHI and particles for 2 – 4 h at 35 – 37 °C.

Following incubation, insert PickPen® tips into the BHI sample wells and transfer the particles to the 2nd set of wells containing Wash Solution and gently swirl for 10 s (do not release particles into solution). Transfer particles to the corresponding row of the prepared resuspension plate using the PickPen®. With tips submerged, retract the PickPen® magnets and tap gently tips to release particles into the Resuspension Buffer Tq. Cover the resuspension plate with adhesive film and continue with step (l).

l. Repeat steps (f) through (k) for all samples using new tips for each strip of samples.

PROCEED TO TEST PROCEDURE SECTION

AOAC® Performance Tested Method 050602

Approved matrices include: Raw Beef Trim, Raw Ground Beef, RTE Poultry Meat (Turkey), Raw Leaf Lettuce, Raw Spinach, Raw Mixed Greens (Baby Lettuce and Other Young Leafy Green Vegetables), Raw Almonds, Raw Strawberries, Stainless Steel and Rubber.

Sample Preparation

A. Enrichment Media Preparation

a) For 25 g (mL) sample, pre-warm 225 mL sterile deionized water at 41 – 43 °C overnight. On day of use, aseptically transfer 7.1 g of BioControl mEHEC® media into the pre-warmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.

b) For 375 g sample, pre-warm 1500 mL sterile deionized water at 41 – 43 °C overnight. On day of use, aseptically transfer 47.3 g of BioControl mEHEC® media into the pre-warmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.

c) Alternatively, mEHEC media can be prepared in advance and autoclaved. Add 31.6 g media per liter of deionized water. Stir to dissolve the powder, dispense into desired volume and autoclave at 121 °C for 15 min. Media must be pre-warmed to 41 – 43 °C overnight prior to sample addition.

B. Test Portion Preparation & Enrichment

a. 25 g samples: Add 25 g of sample to 225 mL pre-warmed (41 – 43 °C) mEHEC media. If necessary, masticate or homogenize sample for 2 min. Incubate for 8 – 24 h at 41 – 43 °C.

b. 375 g samples: Add 375 g of sample to 1500 mL pre-warmed (41 – 43 °C) mEHEC media. If necessary, masticate or homogenize sample for 2 min. Incubate for 10 – 24 h at 41 – 43 °C.

c. For **environmental** monitoring, pre-moisten sterile dehydrated sponges with 10 mL D/E (Dey/Engley) Broth or Letheen Broth. Hydrate sterile swab by soaking in D/E or Letheen broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of pre-warmed (41 – 43 °C) mEHEC media respectively. Masticate sponge and media to mix well. Incubate for 8 – 24 h at 41 – 43 °C.

C. Sample Processing Protocol

Change gloves prior to handling reagents.

a. Vortex **Concentration Reagent**. Immediately transfer 20 µL to each of the required number of Assurance® GDS sample wells (1 well /sample) using a repeat pipette and 0.5 mL pipette tip. Cover sample wells with adhesive film strips.

b. Transfer 1.0 mL of **Wash Solution** to additional sample wells (1 well/sample) using a repeat pipette and 10 mL pipette tip.

c. Transfer 45 µL of **Resuspension Buffer Tq** to the wells in the resuspension plate using a repeat pipette and a 0.5 mL pipette tip. Cover resuspension plate with adhesive film strips.

d. After incubation, carefully remove adhesive film from 1 strip of sample wells. Add 1.0 mL of incubated sample to each sample well containing Concentration Reagent. Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film strip prior to adding samples to a new strip. **Return samples to 42 °C incubator.**

e. Place sealed sample wells on the vortex mixer and vortex at approximately 900 rpm for 10 – 20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.

f. Carefully remove and discard adhesive film strip from a strip of samples. Remove corresponding film strip from a strip of sample wells containing Wash Solution.

g. Load tips onto the PickPen® device, ensuring that the tips are firmly in place on the PickPen® tool. Extend the PickPen® magnets and insert tips into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Gently tap the PickPen® tips against the side of the sample wells to remove excess media droplets.

h. Transfer PickPen® tips to corresponding sample wells containing Wash Solution and gently swirl for 10 s (do not release particles into solution). Transfer PickPen® tips to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen® magnets and tap tips gently to release particles into the Resuspension Buffer Tq.

i. Repeat steps (f) through (h) for all samples using new tips for each strip of samples.

j. Cover resuspension plate with adhesive film strips.

PROCEED TO TEST PROCEDURE SECTION

Health Canada Method MFLP-36

Approved for all foods and environmental surface samples except for the following 2 categories: fruit and vegetable-based products and ready-to-eat meat and poultry products.

Sample Preparation

A. Test Portion Preparation & Enrichment

a. Add 25 g (mL) of sample to 225 mL of Buffered Peptone Water (BPW).

For raw seafood, add 25 g of sample to 225 mL BPW with novobiocin (Appendix A).

For non-fat dry milk, add 25 g of sample to 225 Brilliant Green Water (Appendix A).

For chocolate products, add 25 g of sample to 225 mL of reconstituted nonfat dry milk (Appendix A). Homogenize sample allow sample to sit at room temperature for 1 h and adjust pH to 6.6 – 7.0. Add 0.45 mL of 1% Brilliant Green Dye Solution (1 g Brilliant Green Dye in 100 mL sterile deionized water) and mix.

For poultry rinse, follow Health Canada MFHPB-20 for sample size and enrichment volume.

For environmental monitoring, pre-moisten sterile dehydrated sponges with 10 mL D/E (Dey/Engley) Broth or Letheen Broth. Hydrate sterile swab by soaking in D/E or Letheen broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of BPW, respectively. If alternate test portion sizes are analyzed, proportionately adjust the volume of media to maintain 1:9 ratio.

b. Homogenize or mix sample and incubate samples for 18 – 24 h at 35 – 37 °C. Incubate **non-fat dry milk** samples for 20 – 24 h at 35 – 37 °C.

c. For **high microbial load**, **non-fat dry milk** and **chocolate product** samples, transfer enriched samples to BHI for 2 – 4 h at 35 – 37 °C as described in step **B** (**i**).

Note: Contact us for recommended procedures for testing alternate sample sizes.

B. Sample Processing Protocol

Change gloves prior to handling reagents.

a. Vortex **Concentration Reagent**. Immediately transfer 20 µL to each of the required number of Assurance® GDS sample wells (1 well /sample) using a repeat pipette and 0.5 mL pipette tip. Cover sample wells with adhesive film strips.

> **Note:** Generally, foods with an aerobic plate counts of less than 10^4 CFU/g are considered to have a low microbial load. Raw and high moisture foods are considered high microbial load; processed and low moisture foods are considered low microbial load.

b. For **low microbial load**, **raw seafood** and **environmental surface** samples, transfer 1.0 mL of **Wash Solution** to additional sample wells (1 well/sample) held in a sample well base using a repeat pipette and 10 mL pipette tip. Cover wells with adhesive film strips.

c. For **high microbial load** and **non-fat dry milk** samples, dispense 0.5 mL of sterile Brain Heart Infusion (BHI) broth to sample wells (1 well /sample) in place of Wash Solution. Cover wells with adhesive film strips.

d. For **chocolate product** samples, transfer 1.0 mL of **Wash Solution** to each of 2 additional sample wells (2 wells/sample) using a repeat pipette and 10 mL pipette tips. Cover wells with adhesive film strips. Transfer 0.5 mL of sterile Brain Heart Infusion (BHI) broth to an additional set of sample wells (1 well/sample) using a repeat pipette and 10 mL pipette tips. Cover wells with adhesive film strips.

e. Transfer 45 µL of **Resuspension Buffer Tq** to the wells in the resuspension plate using a repeat pipette and a 0.5 mL pipette tip. Cover resuspension plate with adhesive film strips.

f. Carefully remove adhesive film from 1 strip of sample wells. Add 1.0 mL of incubated sample to each sample well containing Concentration Reagent.

   Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film strip prior to adding samples to a new strip. **Immediately return samples to incubator.**

g. Place sealed sample wells on the vortex mixer and vortex at approximately 900 rpm for 10 – 20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.

h. Carefully remove and discard adhesive film strip from a strip of samples. Remove corresponding film strip from a strip of sample wells containing Wash Solution or BHI.

i. For all samples load tips onto the PickPen® device, ensuring that the tips are firmly in place on the PickPen® tool. Extend the PickPen® magnets and insert tips into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Gently tap the PickPen® tips against the side of the sample wells to remove excess media droplets.

j. For **low microbial load**, **raw seafood** and **environmental surface** samples, transfer PickPen® tips to corresponding sample wells containing Wash Solution and gently swirl for 10 s (do not release particles into solution). Transfer PickPen® tips to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen® magnets and tap tips gently to release particles into the Resuspension Buffer Tq. Cover resuspension plate with adhesive film strips and continue with step (m).

k. For **high microbial load** and **non-fat dry milk** samples, transfer PickPen® tips to corresponding sample wells containing BHI. With tips submerged, retract the PickPen® magnets and tap gently to release particles into the BHI. Cover each strip with a new adhesive film strip prior to adding samples to a new strip. Incubate sample wells containing BHI and particles for 2 – 4 h at 35 – 37 °C.

   Following incubation, transfer the particles from the BHI sample wells to the corresponding row of the prepared resuspension plate using the PickPen®, as indicated in steps B(h) – B(j). With tips submerged, retract the PickPen® magnets and tap tips gently to release particles into the Resuspension Buffer Tq. Cover the resuspension plate with adhesive film and continue with step (m).

l. For **chocolate product** samples, transfer PickPen® tips to 1st set of sample wells containing Wash Solution and gently swirl for 10 s (do not release particles into solution). Next, transfer PickPen® tips to corresponding sample wells containing BHI. With tips submerged, retract the PickPen® magnets and tap

gently to release particles into the BHI. Cover each strip with a new adhesive film strip prior to adding samples to a new strip. Incubate sample wells containing BHI and particles for 2 – 4 h at 35 – 37 °C.

Following incubation, insert PickPen® tips into the BHI sample wells and transfer the particles to the 2nd set of wells containing Wash Solution and gently swirl for 10 s (do not release particles into solution). Transfer particles to the corresponding row of the prepared resuspension plate using the PickPen®. With tips submerged, retract the PickPen® magnets and tap gently tips to release particles into the Resuspension Buffer Tq. Cover the resuspension plate with adhesive film and continue with step (m).

m. Repeat steps (h) through (l) for all samples using new tips for each strip of samples.

PROCEED TO TEST PROCEDURE SECTION

Test Procedure

Change gloves prior to handling reagents.

A. Preparation of Gel Cooling Block

a) Prior to initial use, the gel cooling block must be stored in the freezer (-25 to -15 °C) for 6 h. When frozen the gel cooling block will change color from pink to purple. When not in use the gel cooling block should continue to be stored at -25 to -15 °C.

b) Between each use the gel cooling block should be returned to the freezer until it has turned completely purple, indicating it is ready for use. This may take up to 2 h.

c) The aluminum cooling block is for use with the 576 test kit and should be stored in the refrigerator (2 – 8 °C). To use, place the refrigerated aluminum cooling block on top of the frozen gel cooling block.

B. Preparation of Amplification Tubes

a. The Assurance® GDS Rotor-Gene set up and data entry should be completed prior to transferring samples from the resuspension plate into the Amplification Tubes.

b. Remove **Amplification Tubes Tq** from foil pouch and place them in the frozen gel cooling block (aluminum cooling block for 576 test kit). Reseal pouch.

c. Transfer 30 µL of sample from the resuspension plate wells into each Amplification Tube using a multi-channel pipette and filter barrier tips. Firmly press down on each Amplification Tube lid to close. Visually inspect each tube to ensure that the cap is securely sealed.

d. Place Amplification Tubes into Assurance® Rotor-Gene in sequential order, beginning with position #1. For the 100 test kit, use the 36-well rotor and locking ring; for the 576 test kit, use the 72-well rotor and locking ring.

Note: For 576 test kit, after loading amplification tubes in the rotor and securing with locking ring, contents should be thoroughly mixed by shaking with a snapping motion. See Application Note FRMMK.2060 for details.

e. Start Rotor-Gene cycle. Refer to Assurance® GDS user manual for detailed instructions on operating the Rotor-Gene.

Note: The Assurance® GDS Rotor-Gene must be started within 20 min after addition of the samples to the Amplification Tubes.

Results

Upon completion of the run, the Assurance® GDS Rotor-Gene software will provide a results table. Each sample will be identified as **Positive**, **Negative**, or **No Amp.**

Positive: Samples are positive for *Salmonella*.

Negative: Samples are negative for *Salmonella*.

No Amp: Amplification did not occur. Repeat the test beginning from Step B. Sample Preparation Protocol. If the No Amp result repeats, contact Technical Services (BioMTS@milliporesigma.com).

No.	Color	Name	Result	Assay	Kit Lot Number
1		Sample 1	Positive	*Salmonella*	1234567
2		Sample 2	Negative	*Salmonella*	1234567
3		Sample 3	No Amp	*Salmonella*	1234567

Confirmation

Samples enriched as specified by **AOAC® OMA 2009.03** should be incubated for 20 – 24 h and samples enriched as specified by **AOAC® PTM 050602** should be incubated for 16 – 24 h prior to transfer to a selective enrichment broth for confirmation.

Presumptive positive samples should be confirmed from the retained Assurance® GDS enrichment media via:

US FDA.2014. *Bacteriological Analytical Manual* online, chapter 5.
http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm

USDA – FSIS. 2014. *Microbiology Laboratory Guidebook*, 4.08.

http://www.fsis.usda.gov/wps/wcm/connect/fsis-content/internet/main/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook

Health Canada Method MFLP-36: Presumptive positive results may be confirmed from the primary enrichment broth by proceeding with the plating and confirmation steps of an appropriate reference method such as MFHBP-20. http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume2-eng.php

Storage

Store Assurance® GDS for *Salmonella* Tq kit components at 2 – 8 °C. Kit expiration is provided on the product box label.

Precautions

If possible, maintain separate work zones and dedicated equipment and supplies for sample preparation and amplification and detection.

It is recommended to utilize both positive and negative control samples.

This product is not intended for human or veterinary use. Assurance® GDS for *Salmonella* Tq must be used as described herein. Contents of the test may be harmful if swallowed or taken internally.

Do not use test kit beyond expiration date on the product box label. Decontaminate and dispose of materials in accordance with good laboratory practices and in accordance with local, state and federal regulations.

Do not open or autoclave used Amplification Tubes. After run is complete, place used Amplification Tubes into a sealed container with sufficient volume of a 10% bleach solution to cover tubes for a minimum of 15 min or double bag amplification tubes and dispose outside of the lab.

If contamination is suspected, moisten paper towel with bleach solution and wipe all lab benches and equipment surfaces with 10% bleach solution. Avoid spraying bleach solution directly onto surfaces. Allow bleach solution to remain on surfaces for a minimum of 15 min before wiping clean with 70% isopropyl alcohol solution.

To prepare 10% bleach solution add 10 mL of commercially available bleach containing at least 5% sodium hypochlorite to 90 mL of deionized water. The minimum final concentration of sodium hypochlorite in the bleach solution should be 0.5%. The bleach solution is stable for 7 days from preparation. To prepare 70% isopropyl alcohol solution add 70 mL of pure isopropyl alcohol to 30 mL of deionized water or buy commercially available 70% isopropyl alcohol.

Waste may be contaminated with *Salmonella* which is potentially hazardous to human health. All biohazard waste should be disposed of appropriately.

Appendix A – Enrichment Media Recipes

Buffered Peptone Water

Suspend 20 g of dehydrated Buffered Peptone Water (BPW) in 1 L of deionized water. Mix thoroughly and dispense into desired aliquots. Autoclave at 121 °C for 15 min.

Buffered Peptone Water w/ Novobiocin

Prepare BPW as described above. On day of use, add 1 mL of 0.4% Novobiocin solution to 225 mL BPW.

0.4% Novobiocin Solution

Dissolve 0.4 g of Novobiocin (sodium salt) in 100 mL of sterile deionized water. Store in dark at 2 – 8 °C.

Brain Heart Infusion

Suspend 37 g of Brain Heart Infusion in 1 L of deionized water. Mix thoroughly and dispense into desired aliquots. Autoclave at 121 °C for 15 min.

Brilliant Green Water

Create a 1% Brilliant Green Dye stock solution by dissolving 1 g Brilliant Green Dye in 100 mL of sterile deionized water (Do not autoclave). To prepare Brilliant Green Water, add 2 mL of the 1% Brilliant Green Dye stock solution to 1 L of sterile purified water.

Reconstituted Nonfat Dry Milk

Suspend 100 g dehydrated nonfat dry milk in 1 L deionized water. Autoclave at 121 °C for 15 min.

Tryptic Soy Broth

Suspend 30 g of Tryptic Soy Broth in 1 L of deionized water. Mix thoroughly and dispense into desired aliquots. Autoclave at 121 °C for 15 min.

Manufacturing Entity

BioControl Systems, Inc, 12822 SE 32nd St, Bellevue, WA 98005, USA.

BioControl Systems, Inc is an affiliate of Merck KGaA, Darmstadt, Germany.

MilliporeSigma
400 Summit Drive
Burlington, MA 01803
United States

SigmaAldrich.com/GDS

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