

Assurance® GDS *Cronobacter* Tq II

MicroVal Certificate No. 2017LR77

Part No: 71038-100 (100 tests)
71038-576ATM (576 tests)

General Description

Assurance® GDS for *Cronobacter* Tq II (GDS *Cronobacter*) is an automated nucleic acid amplification system for the detection of *Cronobacter* in infant nutritional formula, ingredients, and environmental samples. Assurance® GDS assays are designed for use by qualified lab personnel who follow appropriate microbiology laboratory practices.

Kit Components

Each Assurance® GDS for *Cronobacter* Tq II 100 kit contains the following:

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

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

- Amplification Tubes Tq
- Concentration Reagent

The following are also necessary for 576ATM kit, 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® thermocycler
- PickPen® device and PickPen® tips
- GDS rotor and locking ring
- Laptop computer and software v2.3.103
- Vortex mixer (IKA® MS 3 or equivalent)
- Adhesive film strips
- Sample wells and sample wells base
- Resuspension plate
- Stomacher® paddle homogenizer, or equivalent
- Stomacher®-type bags with filter, or equivalent

8-channel micropipette capable of accurately dispensing 30 µL

Adjustable micropipette capable of accurately dispensing 20 µL, 45 µL, 0.3 mL, 0.5 mL, 0.7 mL and 1.0 mL

Repeat pipette

Repeat pipette tips (0.5 mL and 10 mL)

Filter-barrier micropipette tips (50 µL and 1.0 mL)

Gel cooling block

Incubator capable of maintaining up to 36 ± 2 °C

Incubator capable of maintaining 41.5 ± 1 °C

Incubator capable of maintaining 44 ± 1 °C

Freezer capable of maintaining -20 ± 5 °C

Refrigerator capable of maintaining 5 ± 3 °C

MICROVAL CERTIFICATE No. 2017LR77

Approved categories include: Infant formula, infant cereal, NFDM, and environmental samples.

TEST PORTION PREPARATION & ENRICHMENT

Please see APPENDIX A for Enrichment Media. See APPENDIX B for Enrichment Method tables.

Note: For this method, when a temperature of 36 °C is specified, the acceptable temperature range is 36 ± 2 °C.

A. Sample Preparation & Enrichment – Infant formula, infant cereals, non-fat dry milk (NFDM), and other infant nutritional ingredients

Note: The media should be pre-warmed to 36 °C before sample addition.

1. Samples without subculture in brain heart infusion (BHI) broth

All samples are at a 1:10 sample-to-total-volume ratio.

- a. **Infant formula without probiotics**, most **non-probiotic ingredients** (except **NFDM**): For 10 – 25 g and 26 – 375 g samples, add to Buffered Peptone Water (BPW, Appendix A).

Infant formula with probiotics: For 10 – 25 g and 26 – 375 g samples, add to BPW with vancomycin (BPW+v, Appendix A). Homogenize or mix enrichments.

Infant cereal without probiotics: For 10 – 25 g and 26 – 375 g samples, add to BPW with amylase (BPW+a, Appendix A). Homogenize or mix enrichments.

Infant cereal with probiotics: For 10 – 25 g and 26 – 375 g samples, add to BPW with vancomycin and amylase (BPW+v+a, Appendix A). Homogenize or mix enrichments.

- b. For powdered or dry products, do not shake; instead allow to stand undisturbed in the media at 18 – 27 °C (laboratory temperature) for 30 – 60 min. If after this time the powder is not homogenized, mix the content till dissolved.
- c. Incubate 10 – 25 g samples for 20 – 28 h at 36 °C. Incubate 26 – 375 g samples for 24 – 32 h at 36 °C.
- d. Continue to SAMPLE PROCESSING PROTOCOL.



2. Samples with subculture in BHI

All samples are at 1:10 sample-to-total-volume ratio.

- NFDM:** For 10 – 25 g and 26– 375 g samples, add to Brilliant Green Water (BGW, Appendix A).
- Do not shake; instead allow to stand undisturbed in the media at 18 – 27 °C (laboratory temperature) for 30 – 60 min. If after this time the powder is not homogenized, mix the content till dissolved.
- Incubate 10 – 25 g samples for 20 – 28 h at 36 °C. Incubate 26 – 375 g samples for 24 – 32 h at 36 °C.
- Transfer enriched samples to BHI (Appendix A) broth for 2 – 4 h at 36 °C as described in C. Sample Processing Protocol, step 10.
- Continue to SAMPLE PROCESSING PROTOCOL.

B. Sample Preparation & Enrichment – Environmental Samples

1. Samples without subculture in BHI

- Environmental monitoring** (stick swabs and sponges): Pre-moisten sterile dehydrated sponges with 10 mL BPW. Hydrate sterile swab by soaking in BPW. If neutralization is needed, substitute D/E (Dey/Engley) Broth or Lethen Broth for the BPW. After collecting sample from surface, add sponge or swab to 100 mL or 10 mL of BPW, respectively. Incubate samples for 20 – 28 h at 36 °C.

Areas of sampling:

- Food (and non-food) product contact surfaces, work surfaces and adjacent areas (e.g., blenders, worktables, drip shields, housing)
 - Non-food contact surfaces not close to food product work surfaces (e.g., drains, floors, walls, wheels of cart)
- Dusts and process water:** Aseptically weigh 25 g sweepings to 225 mL BPW. Aseptically add 25 mL process water to 225 mL of BPW. Incubate samples for 20 – 28 h at 36 °C.

C. Sample Processing Protocol

Change gloves prior to handling reagents.

- Vortex **Concentration Reagent**. Immediately transfer 20 µL to each of the required number of GDS sample wells (1 well/sample) using a repeat pipette and a 0.5 mL pipette tip. Add 0.7 mL of Wash Solution to sample wells containing Concentration Reagent using a repeat pipette and a 10 mL pipette tip. Cover sample wells with adhesive film strips.
- Transfer 1.0 mL of **Wash Solution** to required number of GDS sample wells (1 well/sample) using a repeat pipette and a 10 mL pipette tip. Cover sample wells with adhesive film strips.
For **NFDM** samples, dispense 0.5 mL of sterile BHI broth to required number of GDS sample well (1 well/sample) in place of Wash Solution using a repeat pipette and a 10 mL pipette tip. Cover sample wells with adhesive film strips.
- Transfer 45 µL of **Resuspension Buffer Tq** to the sample wells in the resuspension plate (1 well/sample) using a repeat pipette and a 0.5 mL pipette tip. Cover resuspension plate with adhesive film strips.
- Carefully remove adhesive film from 1 strip of sample wells containing Concentration Reagent and Wash Solution. Add 0.3 mL of incubated sample to each sample well using adjustable micropipette and 1.0 mL filter barrier tips. Avoid transferring food particles. A new pipette tip must be used for each enrichment. Cover each strip of sample wells with a new adhesive film prior to adding samples to a new strip of wells. **Immediately return samples to incubator for confirmation, if necessary.**
- Place sealed sample wells containing Concentration Reagent and enrichments 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.



6. Carefully remove and discard adhesive film from 1 strip of samples. Remove corresponding adhesive film from a strip of sample wells containing Wash Solution or BHI.
7. 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. Tap the PickPen® tips against the side of the sample wells to remove excess media droplets.
8. For all samples **except NFDM**, transfer PickPen® tips to Wash Solution. With tips submerged, gently stir the PickPen® from side to side for 10 – 20 s (do not release particles into solution). Tap the PickPen® tips against the side of the wells to remove excess Wash Solution droplets.
9. Transfer PickPen® tips to the corresponding column 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 the resuspension plate column with adhesive film and continue with step 12.
10. For **NFDM** samples, transfer PickPen® tips to corresponding sample wells containing BHI. With tips submerged, retract the PickPen magnets and tap tips gently to release particles into the BHI. Cover each strip with a new adhesive film prior to adding samples to a new strip of sample wells containing BHI. Incubate sample wells containing BHI and particles for 2 – 4 h at 36 °C.
11. After incubation, transfer the particles from the BHI sample wells to the corresponding column of the prepared resuspension plate using the PickPen® device, as indicated in steps 8 – 9. With tips submerged, retract the PickPen® magnets and tap tips gently to release particles into the Resuspension Buffer Tq. Cover the resuspension plate column with adhesive film.
12. Repeat steps 6 through 11 for all samples using new tips for each strip of samples.

Proceed to Test Procedure Section

Test Procedure (Amplification & Detection)

Change gloves prior to handling reagents.

A. Preparation of Gel Cooling Block

1. Prior to initial use, the gel cooling block must be stored in the freezer (-20 ± 5 °C) for minimum 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 upside-down at -20 ± 5 °C.
2. Between each use, the gel cooling block should be returned to the freezer and stored upside-down until it has turned completely purple, indicating it is ready for use. This may take up to 2 h.

B. Preparation of Amplification Tubes

1. 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**.
2. Remove Amplification Tubes from foil pouch and place them in the frozen gel cooling block. Reseal pouch.
3. Open Amplification Tubes. Transfer 30 µL of sample from resuspension plate 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.
4. Place Amplification Tubes into the Assurance® GDS Rotor-Gene® sequential order, beginning with position #1. Start Rotor-Gene® cycle. Refer to Assurance® GDS User Manual for detailed instructions on operating the Rotor-Gene® thermocycler.

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 thermocycler software will provide a results table. Each sample will be identified as **Positive**, **Negative**, or **No Amp**.

Positive: Samples are positive for *Cronobacter* spp.

Negative: Samples are negative for *Cronobacter* spp.

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

No.	Color	Name	Result	Description	Kit Lot Number
1	■	Sample 1	Positive	<i>Cronobacter</i>	1234567
2	■	Sample 2	Negative	<i>Cronobacter</i>	1234567
3	■	Sample 3	No Amp	<i>Cronobacter</i>	1234567

Note: Enriched samples can be stored at 5 ± 3 °C for up to 72 h prior to testing with GDS *Cronobacter*.

Cultural Confirmation

Enriched samples can be stored at 5 ± 3 °C for up to 72 h prior to confirmation. For **NFDM** samples, store BGW (and not BHI subculture) enrichment at 5 ± 3 °C.

- Confirm typical colonies via ISO 22964 (2017): Horizontal method for the detection of *Cronobacter* spp. Transfer 0.1 mL enrichment broth to 10 mL *Cronobacter* Selective broth (CSB). Incubate CSB broth for 24 ± 2 h at 41.5 ± 1 °C. Streak 10 µL CSB onto Chromogenic *Cronobacter* Isolation (CCI) agar (Merck 1205960500, or equivalent). Incubate plates for 24 ± 2 h at 41.5 ± 1 °C.
- Cronobacter* may be isolated from GDS-positive samples by directly streaking enrichment to a choice of chromogenic agars: CCI agar (Merck 1205960500, or equivalent), Brilliance *Cronobacter sakazakii* agar (DFI, ThermoFisher CM1055, or equivalent), RAPID'Sakazakii agar (RSA, Bio-Rad 3564976, or equivalent). Streak plates for isolation. Incubate CCI plates for 24 ± 2 h at 41.5 ± 1 °C. Incubate DFI plates for 24 ± 2 h at 36 ± 2 °C. Incubate RSA plates for 24 ± 2 h at 44 ± 1 °C.

For **NFDM** samples, isolate only after same-day analysis by GDS and only onto CCI plates.

Confirm colonies using rapid biochemical gallery (e.g., bioMérieux API® 20E / 32E kit), or appropriate ISO 16140-6:2019 validated confirmation method (e.g., Bruker MALDI ToF Biotyper®).

Note: If *Cronobacter* is not isolated using the direct streak method (above), *Cronobacter* may then be isolated using the resuspension plate containing remaining IMS particles targeting *Cronobacter* in resuspension buffer from the GDS-positive samples. See option C, below.

- Cronobacter* may be isolated from GDS-positive samples by plating the GDS concentration reagent which remains in resuspension plate after analysis.
 - From the sample resuspension plate previously used for GDS *Cronobacter* analysis (Test Procedure, Step B), briefly pipette up and down remainder liquid contained in well. This will resuspend the IMS beads contained in Resuspension Buffer Tq (approximately 10-15 µL volume remains).
 - Transfer all volume (10-15 µL) of suspended IMS beads to first quadrant of the choice of chromogenic agar: CCI or DFI plates. Streak plates for isolation. Incubate CCI plates for 24 ± 2 h at 41.5 ± 1 °C. Incubate DFI plates for 24 ± 2 h at 36 ± 2 °C.



3. Confirm colonies using rapid biochemical gallery (e.g., bioMérieux API® 20E/ 32E kit), or appropriate ISO 16140-6:2019 validated confirmation method (e.g., Bruker MALDI ToF Biotyper®).

Note: The original GDS *Cronobacter* sample resuspension plate can be stored at 5 ± 3 °C (refrigeration) for up to 48 h prior to confirmation.

Storage

Store Assurance® GDS for *Cronobacter* Tq II kit components at 5 ± 3 °C.

Precautions

Do not use test kit beyond expiration date on the product box label. Do not use Assurance® GDS for *Cronobacter* Tq II reagents that have expired.

Assurance® GDS for *Cronobacter* Tq II must be used as described herein.

Safety

Assurance® GDS for *Cronobacter* Tq II kit.—This product is not intended for human or veterinary use. Assurance® GDS for *Cronobacter* Tq II must be used as described in the package insert.

The user should read, understand and follow all safety information in the instructions for the Assurance® GDS for *Cronobacter* Tq II Kit. Retain the safety instructions for future reference.

Contents of the test may be harmful if swallowed or taken internally.

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. Decontaminate and dispose of materials in accordance with good laboratory practices and in accordance with local, state, and federal regulations. If contamination is suspected, moisten paper towel with 10% bleach solution and wipe all lab benches and equipment surfaces. 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.

Assurance® GDS Rotor-Gene®.—Improper use of the Assurance® GDS Rotor-Gene may cause personal injuries or damage to the instrument. Some components may pose a risk of personal injury due to excessive heat if improperly handled. For safe use, the instrument must only be operated by qualified laboratory personnel who have been appropriately trained. Servicing of instrument must only be performed by MilliporeSigma/Merck KGaA Service Engineers.

Sample Enrichment.— To reduce the risks associated with exposure to chemicals and biohazards, perform pathogen testing in a properly equipped laboratory under the control of trained personnel. Always follow standard laboratory safety practices, including wearing appropriate personal protective apparel and eye protection, PPE, while handling reagents and contaminated samples. Avoid contact with the contents of the enrichment media and reagent tubes after amplification. Dispose of enriched samples according to current industry standards. Decontaminate and dispose of materials in accordance with good laboratory practices and in accordance with local, state/provincial, and federal regulations.

Cronobacter Precautions— *Cronobacter* is a biosafety level-2 organism. Biological samples, such as enrichments, have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes. Wear appropriate protective equipment which includes, but is not limited to, protective eyewear, face shield, clothing/laboratory coat, and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment (e.g., physical containment devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements.



before working with potentially infectious materials. All enrichment broths should be sterilized following any culture-based confirmatory steps. Clean the work stations and laboratory equipment with a disinfectant of choice before and after lab activities (e.g., sodium hypochlorite solution, phenol solution, quaternary ammonium solution, etc.).

APPENDIX A – Enrichment Media Recipes

Buffered Peptone Water (BPW)

Follow the manufacturer's instructions for preparation of media.

Buffered Peptone Water w/ Vancomycin (BPW+v)

Prepare BPW as described above.

For 1:10 ratio, on day of use, add 0.675 mL of 0.2% Vancomycin solution to 225 mL BPW (10.1 mL of Vancomycin solution to 3375 mL BPW).

Buffered Peptone Water w/ Amylase (BPW+a)

Prepare BPW as described above.

For 1:10 ratio, on day of use, add 2.25 mL of 1% Amylase solution to 225 mL BPW (33.8 mL of Amylase solution to 3375 mL BPW).

Buffered Peptone Water w/ Vancomycin and Amylase (BPW+v+a)

Prepare BPW as described above.

For 1:10 ratio, on day of use, add 0.675 mL of 0.2% Vancomycin solution and 2.25 mL of 1% Amylase solution to 225 mL BPW (10.1 mL of Vancomycin solution and 33.8 mL of Amylase solution to 3375 mL BPW).

0.2% Vancomycin Solution

Dissolve 0.2 g of Vancomycin (hydrochloride) in 100 mL of sterile deionized water. Store in dark at 5 ± 3 °C.

1% Amylase Solution

Dissolve 1.0 g of alpha-Amylase (~50 U/mg) in 100 mL of sterile deionized water. Store in dark at 5 ± 3 °C.

Note: verify amylase source does not also contain dextrin and is from bacterial origin. We recommend Sigma #10070 or MP Biologics #0210044725.

Brain Heart Infusion (BHI) broth

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

1% Brilliant Green Dye Solution

Dissolve 1 g of Brilliant Green dye in 100 mL of sterile deionized water (do not autoclave). Store in dark at 5 ± 3 °C.

Brilliant Green Water (BGW)

For 1:10 ratio, on day of use, add 0.45 mL of the 1% Brilliant Green Dye solution to 225 mL of sterile deionized water (6.75 mL of 1% Brilliant Green Dye stock solution to 3375 mL of sterile deionized water).



APPENDIX B – *Cronobacter* Enrichment Methods

Table A: Sample Type and Enrichment Method for Assurance® GDS for *Cronobacter* II (Infant Formula, Infant Cereals and Ingredients)

Food Type	Media	Sample Size	Sample:Total Volume Ratio	Enrichment Time	Incubation Temperature
No BHI subculture					
Infant Formula <u>without</u> Probiotics	BPW	10 – 25 g	1:10	20 – 28 h	36 ± 2 °C
		26 – 375 g	1:10	24 – 32 h	36 ± 2 °C
Infant Cereals <u>without</u> Probiotics	BPW+a	10 – 25 g	1:10	20 – 28 h	36 ± 2 °C
		26 – 375 g	1:10	24 – 32 h	36 ± 2 °C
Non-probiotic Ingredients (except NFDM)	BPW	10 – 25 g	1:10	20 – 28 h	36 ± 2 °C
		26 – 375 g	1:10	24 – 32 h	36 ± 2 °C
Infant Formula <u>with</u> Probiotics	BPW+v	10 – 25 g	1:10	20 – 28 h	36 ± 2 °C
		26 – 375 g	1:10	24 – 32 h	36 ± 2 °C
Infant Cereals <u>with</u> Probiotics	BPW+v+a	10 – 25 g	1:10	20 – 28 h	36 ± 2 °C
		26 – 375 g	1:10	24 – 32 h	36 ± 2 °C
With BHI subculture					
NFDM	BGW	10 – 25 g	1:10	20 – 28 h	36 ± 2 °C
		26 – 375 g	1:10	24 – 32 h	36 ± 2 °C

Table B: Sample Type and Enrichment Method for *Cronobacter* II (Environmental Monitoring)

Environmental Sample	Media	Sample Size	Sample: Total Volume Ratio	Enrichment Time	Incubation Temperature
Environmental samples (Nearby food contact surfaces*) (Distant non-food contact surfaces**)	BPW	Swab Sponge	10 mL 100 mL	20 – 28 h	36 ± 2 °C
Process water	BPW	25 mL	1:10	20 – 28 h	36 ± 2 °C
Dust, sweepings	BPW	25 g	1:10	20 – 28 h	36 ± 2 °C

* Food (and non-food) product contact surfaces, work surfaces and adjacent areas (e.g., blenders, worktables, drip shields, housing)

**Non-food contact surfaces not close to work surfaces (e.g., drains, floors, walls, wheels on cart)

Manufacturing Entity

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