

Assurance® GDS *Cronobacter* Tq II

MicroVal Certificate No. 2017LR77

Part No: 71038-100 (100 tests)

General Description

Assurance® GDS for *Cronobacter* Tq II is an automated nucleic acid amplification system for the detection of *Cronobacter* in infant nutritional formula, ingredients, and environmental samples.

Kit Components

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

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

Equipment / Materials Required

Other necessary materials not provided include:

- Media per Appendix A
- Assurance® GDS Rotor-Gene®
- Computer and software
- 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
- Adjustable pipette capable of accurately dispensing 20 µL, 45 µL, 0.3 mL, 0.5 mL, 0.7 mL and 1.0 mL
- Repeat pipette tips (0.5 mL and 10 mL)
- Filter barrier micropipette tips (50 µL and 1.0 mL)
- Incubator capable of maintaining up to 36 ± 2 °C

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Approved categories include: Infant formula, infant cereal, NFDm and Environmental samples.

TEST PORTION PREPARATION & ENRICHMENT

See Appendix B for Enrichment Method Tables

Sample Preparation & Enrichment – Infant formula, infant cereals, NFDM and other infant nutritional ingredients

For powdered or dry products, do not shake, instead allow to stand undisturbed at 18 – 27 °C (laboratory temperature) for 30 min – 60 min. If after this time the powder is not homogenized, mix the content till dissolved.

Note: The media should be pre-warmed to 37 °C before sample addition.

1. Samples without subculture in BHI

- a. **Infant formula without probiotics, most non-probiotic ingredients** (except **NFDM**): For 10- 125 g of sample, add to 1:10 samples to media ratio Buffered Peptone Water (BPW, Appendix A). For 26 – 375 g sample add to 1:10 sample to media ratio (BPW).

Infant formula with probiotics: For 10- 25 g of sample, add to 1:10 samples to media ratio BPW with vancomycin (BPW+v, Appendix A). For 26 – 375 g sample add to 1:10 sample to media ratio (BPW+v).

Infant cereals without probiotics: For 10- 25 g of sample, add to 1:10 samples to media ratio of BPW with amylase (BPW+a, Appendix A). For 26 – 375 g sample add to 1:10 sample to media ratio (BPW+a).

Infant cereal with probiotics: For 10- 25 g of sample, add to 1:10 samples to media ratio of BPW with vancomycin and amylase (BPW+v+a, Appendix A). For 26 – 375 g sample add to 1:10 sample to media ratio (BPW+v+a).

- b. Homogenize or mix sample. Incubate 10 – 25 g samples for 20 – 28 h at 36 ± 2 °C. Incubate 26 – 375 g samples 24 – 32 h at 36 ± 2 °C.
- c. Continue to SAMPLE PROCESSING PROTOCOL.

2. Samples with subculture in BHI

- a. Non-fat dry milk (NFDM): For 10- 25 g of sample, add to 1:10 samples to media ratio of Brilliant Green Water (BGW, Appendix A). For 26– 375 g sample add to 1:10 sample to media ratio (BGW).
- b. Homogenize or mix sample. Incubate 10 – 25 g samples for 20 – 28 h at 36 ± 2 °C. Incubate 26 – 375 g samples 24 – 32 h at 36 ± 2 °C.
- c. Transfer enriched samples to Brain Heart Infusion (BHI, Appendix A) broth for 2 – 4 h at 36 ± 2 °C as described in Sample Processing Protocol (i).
- d. Continue to SAMPLE PROCESSING PROTOCOL.

A. Sample Preparation & Enrichment – Environmental Samples

1. Samples without subculture in BHI.

- a. **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 Letheen Broth for the BPW. After collecting sample from surface, add sponge or swab to 100 mL or 10 mL of BPW, respectively.

Areas of sampling:

- Food (and non-food) product contact surfaces, work surfaces and adjacent areas (i.e. blenders, work tables, drip shields, housing)
- Non-food contact surfaces not close to food product work surfaces (i.e. drains, floors, walls, cart wheels)

(1) Incubate samples for 20 – 28 h at 36 ± 2 °C.

- b. Dusts and process water: aseptically weigh 25 g sweepings to 225 mL BPW. Aseptically add 25 mL process water to 225 mL of BPW.

(1) Incubate samples for 20 – 28 h at 36 ± 2 °C.

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. Add 0.7 mL of Wash Solution to sample wells containing Concentration Reagent using a repeat pipette and 10 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) held in a sample well base using a repeat pipette and 10 mL pipette tip. Cover sample wells with adhesive film strips.

For **non-fat dry milk** samples, dispense 0.5 mL of sterile BHI broth to sample well (1 well/sample) in place of Wash Solution using a repeat pipette and 10 mL pipette tip. Cover 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. Carefully remove adhesive film from 1 strip of sample wells. Add 0.3 mL of incubated sample to each sample well containing Concentration Reagent and Wash Solution using adjustable micropipette and 1.0 mL filter barrier tips.

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 of wells. **Immediately return samples to incubator for confirmation if necessary.**

- 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 1 strip of samples. Remove corresponding film strip from a strip of sample wells containing Wash Solution or BHI.
- g. 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.
- h. For all samples **except non-fat dry milk**, transfer PickPen® tips to corresponding sample wells containing Wash Solution and gently swirl for 10 s (do not release particles into solution). Tap the PickPen® tips against the side of the wells to remove excess Wash Solution droplets. 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 the resuspension plate row with adhesive film and continue with step (j).
- i. For **non-fat dry milk** 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 strip prior to adding samples to a new strip. Incubate sample wells containing BHI and particles for 2 – 4 h at 36 ± 2 °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 C(g) – C(h). 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 (j).

- j. Repeat steps C(g) through C(i) 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 (-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.

- 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.

B. Preparation of Amplification Tubes Tq

- The Assurance® GDS Rotor-Gene® set up and data should be completed prior to transferring samples from the resuspension plate into the **Amplification Tubes Tq**.
- Remove Amplification Tubes Tq from foil pouch and place them in the frozen gel cooling block. Reseal pouch.
- Transfer 30 µL of sample from resuspension plate well 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.
- Place Amplification Tubes into the Assurance® GDS Rotor-Gene® sequential order, beginning with position #1.
- Start Rotor-Gene®. 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 rotary 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 Step **C. Sample Processing Protocol**. If the No Amp result repeats, contact BioControl Technical Service.

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

Confirmation

Enriched samples can be stored at 2 – 8 °C (refrigeration) for up to 72 h prior to testing with Assurance® GDS for *Cronobacter* Tq II. For **non-fat dry milk** samples, store BGW (and not BHI subculture) enrichment at 2 – 8 °C.

If a confirmation of the positive GDS result is needed, following completion of GDS assay run, 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, incubate plates for 24 ± 2 h at 41.5 ± 1 °C. Confirm typical colonies via ISO 22964 (2017): Horizontal method for the detection of *Cronobacter* spp.

As an alternative confirmation method, presumptive positive enrichment broth may be directly streaked on CCI agar, incubate plates for 24 ± 2 h at 41.5 ± 1 °C.

Storage

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

Precautions

Comply with Good Laboratory Practice (refer to EN ISO 7218 standard)

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

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.

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 *Cronobacter* which is potentially hazardous to human health. All biohazard waste should be disposed of appropriately.

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. 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. 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. 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 2 – 8 °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 2 – 8 °C.

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

Brain Heart Infusion

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

Brilliant Green Water (BGW)

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 0.45 mL of the 1% Brilliant Green Dye stock solution to 225mL 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 *Cronobacter* II (Infant Formula, Infant Cereals and Ingredients)

Food Type	Media	Sample size	Sample:Media Ratio	Enrichment Time
No BHI subculture				
Infant Formula <u>without</u> Probiotics	BPW	10 –25 g	1:10	20 – 28 h
		26 – 375 g	1:10	24 – 32 h
Infant Cereals <u>without</u> Probiotics	BPW+a	10 – 25 g	1:10	20 – 28 h
		26 – 375 g	1:10	24 – 32 h
Non-probiotic Ingredients (except nonfat dry milk)	BPW	10 – 25 g	1:10	20 – 28 h
		26 – 375 g	1:10	24 – 32 h
Infant Formula <u>with</u> Probiotics	BPW+v	10 – 25 g	1:10	20 – 28 h
		26 – 375 g	1:10	24 – 32 h
Infant Cereals <u>with</u> Probiotics	BPW+v+a	10 – 25 g	1:10	20 – 28 h
		26 – 375 g	1:10	24 – 32 h
With BHI subculture				
Nonfat dry milk (NFDm)	BGW	10 – 25 g	1:10	20 – 28 h
		26 – 375 g	1:10	24 – 32 h

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

Environmental Sample	Media	Sample size	Sample:Media Ratio	Enrichment Time
Environmental samples (Nearby food contact surfaces*) (Distant non-food contact surfaces**)	BPW	Swab Sponge	10 mL 100 mL	20 – 28 h
Process water	BPW	25 mL	1:10	20 – 28 h
Dust, sweepings	BPW	25 g	1:10	20 – 28 h

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

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

Manufacturing Entity

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