



For Professional Use Only

AmpliSens[®] *Cronobacter sakazakii*-FRT

PCR kit

Instruction Manual

AmpliSens[®]



Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040



Federal Budget Institute of
Science "Central Research
Institute for Epidemiology"
3A Novogireevskaya Street
Moscow 111123 Russia

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1. INTENDED USE

AmpliSens® *Cronobacter sakazakii*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Cronobacter sakazakii* DNA in clinical material by using real-time hybridization-fluorescence detection.



The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Cronobacter sakazakii DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Cronobacter sakazakii* primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® *Cronobacter sakazakii*-FRT** PCR kit is a qualitative test that contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **AmpliSens® *Cronobacter sakazakii*-FRT** PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by a chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *Cronobacter sakazakii*-FRT PCR kit is produced in 1 form:

AmpliSens® *Cronobacter sakazakii*-FRT PCR kit variant FRT-50 F (for use with RG, iQ)

REF R-B58(RG,iQ)-CE.

AmpliSens® *Cronobacter sakazakii*-FRT PCR kit variant FRT-50 F includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FL <i>Cronobacter sakazakii</i> / STI	colorless clear liquid	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Positive Control DNA <i>Cronobacter sakazakii</i> / STI (C+)	colorless clear liquid	0.1	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube

* must be used in the extraction procedure as Negative Control of Extraction.

** add 10 µl of Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (DNA-sorb-B, **REF** K1-2-50-CE or RIBO-prep, **REF** K2-9-Et-50-CE).

AmpliSens® *Cronobacter sakazakii*-FRT PCR kit is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (Qiagen, USA), Q5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent).
- Disposable polypropylene microtubes for PCR (0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5 % sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact, immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.



Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING



Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.



The clinical material must be taken according to state and local authorities' requirements.

AmpliSens® Cronobacter sakazakii-FRT PCR kit is intended for analysis of DNA

extracted by using DNA extraction kits from samples of primary enrichment media (selective liquid media used for detection of *Cronobacter sakazakii*, such as Kessler's medium with glucose, glucose broth with brilliant green and bile or MacConkey broth) prepared in accordance with effective regulatory documents.

7. WORKING CONDITIONS

AmpliSens® *Cronobacter sakazakii*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits:

- DNA-sorb-B, **REF** K1-2-50-CE.
- RIBO-prep, **REF** K2-9-Et-50-CE.



Extract DNA according to the manufacturer's instructions.

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.



Reaction mixture components should be mixed just before analysis with calculating for the required reaction number (test and control samples) according to Appendix 1. Note that even for analysis of one test or control DNA sample it is necessary to run all controls of the PCR amplification stage: positive control (C+) and negative control of amplification (NCA). It is recommended to mix the reagents for an even reaction number to ensure more exact dosage.

1. Before starting work, thaw and thoroughly vortex all reagents of the kit. Make sure that there are no drops on the caps of the tubes.
2. Prepare the required number of tubes including controls. The type of tubes depends on the PCR instrument used for analysis.
3. To prepare the reaction mixture and mixture for two Background tubes mix **PCR-mix-1-FL *Cronobacter sakazakii* / STI**, **PCR-mix-2-FRT** and **Polymerase (TaqF)** in a new sterile tube (see Appendix 1). Add the necessary quantity of polymerase (TaqF) into the remaining reaction mixture (see Appendix 1). Vortex thoroughly.
4. Transfer **15 µl** of the prepared reaction mixture to each PCR tube.
5. Add **10 µl** of **DNA samples** obtained from the clinical samples. Dispose of the unused reaction mixture.



Avoid transferring sorbent beads together with the DNA sample in case of extraction by DNA-sorb-B reagents kit.

6. Carry out the control amplification reactions:

- C+ -Add **10 µl** of **Positive Control DNA *Cronobacter sakazakii* / STI** to the tube labeled C+ (Positive Control of Amplification).
 NCA -Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).



Insert tubes and run amplification program immediately (10–15 min after mixing the reaction mixture with DNA and controls).

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines [2].

1. Create a temperature profile on your instrument as follows:

Table 1

AmpliSens-1 amplification program

Step	Rotor-type instruments ¹			Plate-type instruments ²		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
Hold	95	15 min	1	95	15 min	1
Cycling	95	10 s	45	95	10 s	45
	60	25 s <i>fluorescent signal detection</i>		60	25 s <i>fluorescent signal detection</i>	
	72	10 s		72	10 s	

Fluorescent signal is detected in the channels designed for the FAM/Green and JOE/Yellow/HEX fluorophores on the 2nd step (60°C) of stage Cycling.

- Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin*.
- Insert tubes into the reaction module of the device.
- Run the amplification program with fluorescence detection.
- Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Internal Control DNA is detected in the FAM/Green fluorescence channel, *Cronobacter sakazakii* DNA is detected in the JOE/Yellow/HEX fluorescence channel.

¹ For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.

² For example, iCycler, iQ5, Mx3000P, Mx3000, or equivalent.

See **Guidelines** for data analysis settings for the instrument.

9.1. Interpretation of results

The results are interpreted by the software of the used instrument by the crossing (or not-crossing) of the fluorescence curve with the threshold line.

The principle of interpretation is given in Table 2.

Table 2

Interpretation of amplification results

Ct value in channel		Interpretation
FAM	HEX	
< boundary values* or defined	< boundary values*	<i>Cronobacter sakazakii</i> DNA is detected in a sample
< boundary values*	> boundary values* or undefined	<i>Cronobacter sakazakii</i> DNA is not detected in a sample
> boundary values* or undefined	> boundary values* or undefined	Invalid result

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (Table 3).

Table 3

Results for controls

Control	Stage for control	Result of automatic interpretation		Interpretation
		FAM/Green channel	JOE/Yellow/HEX channel	
C-	DNA extraction	≤ boundary values*	> boundary values* or undefined	OK
NCA	Amplification	> boundary values* or undefined	> boundary values* or undefined	OK
C+	Amplification	< boundary values*	< boundary values*	OK

*For boundary values, see the Important Product Information Bulletin

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

- If the Ct value for the Positive Control of amplification (C+) in the JOE/Yellow/HEX channel is greater than the boundary Ct value, PCR and detection should be repeated for all samples where *Cronobacter sakazakii* DNA was not detected.
- If the Ct value for the Negative Control of extraction (C-) in the JOE/Yellow/HEX channel and/or the Negative Control of amplification (NCA) in the FAM/Green and/or JOE/Yellow/HEX channels is less than the boundary Ct value, PCR analysis should be repeated (starting from DNA extraction stage) for all samples where *Cronobacter sakazakii* DNA was detected.

- Positive result obtained for Negative Control of extraction (C-), that is a sterile sample of the culture medium, may indicate contamination of the primary enrichment medium with the genetic material of the examined microorganism. In this case, the analysis should be repeated. To do this, start from primary enrichment of food with non-contaminated media and perform an additional negative control extraction reaction using the Negative Control (C-) reagent (see Section 3. Content).
- If the Ct value is absent in both JOE/Yellow/HEX and FAM/Green channels or the Ct value in the JOE/Yellow /HEX channel is higher than the specified boundary value, PCR should be repeated. If the same result is obtained, the extraction stage for the sample should be repeated. If the IC signal of this sample was detected normally in any other PCR test, it is not necessary to repeat the extraction stage (if iCycler iQ or iQ5 instruments are used).
- If the Ct value is present for C- in the FAM/Green channel and/or for NCA in the FAM/Green, JOE/Yellow/HEX channels in the results grid, it indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. Test analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.
- If no signal is detected for the positive controls of amplification, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components has not complied with the manufacturer's instruction, or that the reagent kit has expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated.
- If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if iCycler iQ or iQ5 instruments are used).

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® *Cronobacter sakazakii*-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Cronobacter sakazakii*-FRT** PCR kit (except for PCR-mix-1-FL *Cronobacter sakazakii* / STI, Polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *Cronobacter sakazakii*-FRT** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.



PCR-mix-1-FL *Cronobacter sakazakii* / STI, polymerase (TaqF), and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use.



PCR-mix-1-FL *Cronobacter sakazakii* / STI is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Cronobacter sakazakii*-FRT** PCR kit is the following:

Test material	Nucleic acid extraction kit	Sensitivity, GE/ml ³
Kessler's medium with glucose ⁴	DNA-sorb-B	1x10 ³
	RIBO-prep	1x10 ³

13.2. Specificity

The analytical specificity of **AmpliSens® *Cronobacter sakazakii*-FRT** PCR kit is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. Nonspecific reactions were absent while testing human DNA samples and DNA panel of the following microorganisms: 3 strains of *Cronobacter sakazakii*, 4 strains of *Enterobacter cloacae*, 2 strains of *Enterobacter aerogenes*, 2 strains of *Pantoea agglomerans*, 8 strains of *Campylobacter* spp. (*C. jejuni*, *C. coli* and *C. fetus fetus*), 31 strains of different serogroups of *Escherichia coli* (including *EHEC*, *EPEC*, *ETEC*, *EAggEC* and *EIEC*), 18 strains of different serogroups of *Salmonella* spp, 12 strains of different species and serogroups of *Shigella* spp., 22 strains of different species and serogroups of *Yersinia* spp., *Citrobacter freundii*, *Clostridium*

³ Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the medium specified.

⁴ Pretreatment is not required.

perfringens, *Klebsiella pneumonia*, *Listeria monocytogenes*, *Protrus mirabilis*, *Pseudomonas aeruginosa*, *Serratia marcessens*. The clinical specificity of **AmpliSens®** ***Cronobacter sakazakii*-FRT** PCR kit was confirmed in laboratory clinical trials.














14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.
2. Guidelines “Real-Time PCR Detection of *Cronobacter sakazakii* DNA”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens®** ***Cronobacter sakazakii*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

	Catalogue number		Sufficient for
	Batch code		Expiration Date
	<i>In vitro</i> diagnostic medical device		Consult instructions for use
	Version		Keep away from sunlight
	Temperature limitation	NCA	Negative control of amplification
	Manufacturer	C-	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
	Authorised representative in the European Community	IC	Internal control
	Caution	FBIS CRIE	Federal Budget Institute of Science "Central Research Institute for Epidemiology"

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
17.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"