













Salmonella species 225/670 IC

Key to symbols used

	List Number		Store at 2-8°C
	For <i>in Vitro</i> Diagnostic Use		Caution!
	Lot Number		Version
	Expiration Date		Consult instructions for use
	Negative Control		Positive Control
	Contains reagents		Manufacturer

NAME

Salmonella species 225/670 IC

INTENDED USE

Salmonella species 225/670 IC is an *in vitro* nucleic acid amplification test for qualitative detection of *Salmonella species* in the biological materials.

PRINCIPLE OF ASSAY

Salmonella species 225/670 IC test is based on three major processes: sample preparation, nucleic acid amplification of DNA using specific *Salmonella species* primers and detection of the amplified products on agarose gel. The kit contains the Internal Control which may be used in the isolation procedure and serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition.

MATERIALS PROVIDED

- **PCR-mix-1** 55 ready-to-use single-dose test tubes;
 - **PCR-mix-2**, 0,6 ml;
 - **Mineral Oil**, 2,0 ml;
 - **Positive Control C+**, 0,2 ml;
 - **Negative Control C-**, 1,6 ml;
 - **Internal Control IC****, 0,5 ml;
 - **DNA-buffer**, 0,5 ml;
- Contains reagents for 55 tests.

*can be used in the isolation procedure as Negative Control of Extraction.

**add 10 µl of Internal Control during the DNA isolation procedure directly to the sample/lysis mixture (see DNA-Sorb-B ([REF](#) K-1-1/B) protocol)


MATERIALS REQUIRED BUT NOT PROVIDED

- Thermalcycler
- Workstation
- Pipettors with aerosol barrier
- Tube racks

Reagents non provided

- DNA extraction kit (recommended nucleic acid extraction kit: DNA-Sorb-B (Sacace, [REF](#) K-1-1/B))
- Detection agarose kit

WARNINGS AND PRECAUTIONS

-  Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.
- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
- Do not pipette by mouth.
- 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 specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with Biosafety Level 2 or other appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucose membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- This kit is designed for use with "DNA-Sorb-B" extraction kit. It is the user's responsibility if kits other than "DNA-Sorb-B" are used to perform this DNA extraction.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

STORAGE INSTRUCTIONS

Salmonella species must be stored at 2-8°C.

STABILITY

Salmonella species is stable up to the expiration date indicated on the kit label.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Salmonella species can analyze DNA extracted with DNA-Sorb-B (REF K-1-1/B) from:

- whole blood collected in EDTA tubes;
- tissue (≈1,0 gr) homogenized with mechanical homogenizer or scalpel, glass sticks, teflon pestles and dissolved in 1,0 ml of saline water or PBS sterile. Vortex vigorously and incubate 30 min at room temperature. Transfer the supernatant into a new 1,5 ml tube;
- feces:
 - Prepare required quantity of 1,5 ml polypropylene tubes with 0,8 ml of Saline Solution. Add to each tube 0,1 g of feces. Vortex to get the homogeneous suspension and centrifuge for 5 min to 7000-12000g and using a micropipette with a plugged aerosol barrier tip transfer in a new tube 0,1 ml of the bacterial fraction (white-yellowish line between the sediment and the supernatant). Add 0,8 ml of sterile Saline Solution.
 - Vortex vigorously and centrifuge for 5 min at 7000-12000 g. Remove and discard the supernatant.
 - Resuspend the pellet in 0,3 ml of Saline Solution.

It is recommended to process samples immediately after collection. Store samples at 2-8 °C for no longer than 24 hours, or freeze at -20/80°C. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

AMPLIFICATION

1. Prepare required quantity of tubes PCR-mix-1.
2. Pipette 10 µL of PCR-mix-2 and a drop of Mineral oil (20 µl) into each PCR-mix-1 tube.
3. Add to appropriate tube 10 µL of DNA sample obtained after sample preparation.
4. Add 10 µL of DNA-buffer to the tube for Negative Control of Amplification.
5. Add 10 µL of Positive Control to the tube for Positive Control of Amplification.
6. Close PCR-mix-1 tubes and transfer them into the thermocycler only when temperature reaches 95°C and start the following program:

Step	Thermocyclers with active temperature adjustment "GeneAmp PCR System 2400" (Applied Biosystems), "Palm Cycler" (Corbett Research) and oth			Thermocyclers with active temperature adjustment "GeneAmp PCR System 2700" (Applied Biosystems)			Thermocyclers with block temperature adjustment: "Biometra", "MiniCycler", "PTC-100"(MJ Research)		
	°C	Time	Cycles	°C	Time	Cycles	°C	Time	Cycles
0	95°C	Pause		95°C	Pause		95°C	Pause	
1	95°C	2 min	1	95°C	2 min	1	95°C	2 min	1
2	95°C	10 sec	42	95°C	15 sec	42	95°C	1 min	42
	65°C	10 sec		65°C	25 sec		65°C	1 min	
	72°C	10 sec		72°C	25 sec		72°C	1 min	
3	72°C	1 min	1	72°C	1 min	1	72°C	1 min	1
4	10°C	Storage		10°C	Storage		10°C	Storage	

RESULTS ANALYSIS

Analysis of PCR results is based on the presence or absence of specific bands of amplified DNA in Agarose gel (2%). The length of specific amplified DNA fragments is:

- Salmonella species – 225 bp
- Internal Control – 670 bp

RESULTS INTERPRETATION

Table 2. Results for controls

Control	Which step of test is controlled	Specific bands in the gel 225 bp	Specific bands in the gel 670 bp	Interpretation
NCS	DNA isolation	No	Yes	Valid result
DNA-buffer	Amplification	No	No	Valid result
C+	Amplification	Yes	No	Valid result

- Ensure that the Negative Controls (NCS and C-) values for the run are valid. If the run is invalid (contamination – detection of the band of 225 bp or defective extraction/amplification – absence of the band of 670 bp corresponding to Internal Control), the entire test protocol (sample preparation, amplification and detection) should be repeated. Discard any reagents that may be suspect.
- The sample is considered to be positive for Salmonella species DNA if the band of 225 bp is observed on agarose gel.

PERFORMANCE CHARACTERISTICS

Analytical specificity

The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific Salmonella species primers and probes. The specificity of the kit Salmonella species 225/670 IC was 100%. The potential cross-reactivity of the kit Salmonella species 225/670 IC was tested against the group control. It was not observed any cross-reactivity with other pathogens.

Analytical sensitivity

The kit Salmonella species 225/670 IC allows to detect Salmonella species DNA in 100% of the tests with a sensitivity of not less than 500 copies/ml. The detection was carried out on the control standard and its dilutions by negative sample.

Target region: Fim A gene

