











Mycobacterium paratuberculosis 209/700 IC

Key to symbols used

	List Number		Store at 2-8°C
	Research use only		Caution!
	Lot Number		Version
	Expiration Date		Consult instructions for use
	Contains reagents		Manufacturer

NAME

Mycobacterium paratuberculosis 209/700 IC

INTENDED USE

Mycobacterium paratuberculosis 209/700 IC is nucleic acid amplification test for qualitative detection of *Mycobacterium avium subsp. paratuberculosis*.

PRINCIPLE OF ASSAY


Mycobacterium paratuberculosis 209/700 IC Test is based on three major processes: sample preparation, nucleic acid amplification of DNA using specific *Mycobacterium Paratuberculosis* primers and detection of the amplified products on agarose gel.

MATERIALS PROVIDED

- **PCR-mix-1**, 55 ready-to-use single-dose test tubes;
- **PCR-mix-2**, 0,6 ml;
- **Positive Control C+** (DNA *M. Paratuberculosis*), 0,2 ml (red cap);
- **Negative Control C-**, 3 x 1,6 ml;*
- **Internal Control IC**, 1,0 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  K-1-1/B) protocol)

MATERIALS REQUIRED BUT NOT PROVIDED

- Thermalcycler
- Workstation
- Pipettors (capacity 0,5-10 µl; 5-40 µl) with aerosol barrier
- Tube racks

Reagents non provided

- DNA extraction kit (recommended nucleic acid extraction kit: DNA-Sorb-B (Sacace,  K-1-1/B)
- Detection agarose kit (Precast gel 2%  G1-1)

WARNINGS AND PRECAUTIONS

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
4. Do not use a kit after its expiration date.
5. Dispose of all specimens and unused reagents in accordance with local regulations.
6. Specimens should be considered potentially infectious and handled in biological cabinet in accordance with Biosafety Level 2 or other appropriate biosafety practices.
7. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
8. 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.
9. Material Safety Data Sheets (MSDS) are available on request.
10. 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.
11. Use of this product should be limited to personnel trained in the techniques of DNA amplification.
12. 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

Mycobacterium paratuberculosis 209/700 IC must be stored at 2-8°C.

STABILITY

Mycobacterium paratuberculosis 209/700 IC is stable up to the expiration date indicated on the kit label.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Mycobacterium paratuberculosis 209/700 IC can analyze DNA extracted with **DNA-Sorb-B** (REF K-1-1/B) from

- feces:
 - Prepare 20% feces suspension by adding in 5 ml tube of 4ml of Saline Solution and 1,0 gr (approx. 1,0 ml) of feces. Vortex to get the homogeneous suspension. Allow to settle at room temperature for 5 minutes. Put 1,0 ml of supernatant in a new sterile 1,5 ml tube previously labeled.
 - Centrifuge for 5 min to 600-800g. Use 50 µl of supernatant for DNA extraction.
- tissue samples homogenized with mechanical homogenizer and dissolved in PBS sterile

Specimens can be stored at +2-8°C for no longer than 48 hours, or freeze at -20°C to -80°C.

Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

AMPLIFICATION

- Prepare required quantity of tubes **PCR-mix-1**.
- Pipette **10 µL** of **PCR-mix-2** into each PCR-mix-1 tube.
- Add to appropriate tube **10 µL** of **DNA** sample obtained after sample preparation.
- Add **10 µL** of **DNA-buffer** to the tube for Negative Control of Amplification.
- Add **10 µL** of **Positive Control** to the tube for Positive Control of Amplification.
- Close PCR-mix-1 tubes and transfer them into the thermalcycler only when temperature reaches 95°C and start the following program

Step	t°C	Time	Cycles
0	95°C	Pause	
1	95°C	2 min	1
2	95°C	1 min	42
	66°C	1 min	
	72°C	1 min	
3	72°C	1 min	1
4	10°C	Storage	

RESULTS ANALYSIS

Analysis of 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:

- Mycobacterium Paratuberculosis* – **209 bp**
- Internal Control* – **700 bp**

RESULTS INTERPRETATION

Table 2. Results for controls

Control	Which step of test is controlled	Specific bands in the gel 209 bp	Specific bands in the gel 700 bp	Interpretation
NC	DNA isolation	-	+	Valid result
C-	Amplification	-	-	Valid result
C+	Amplification	+	-	Valid result

- Ensure that the Controls (NC, C-, C+) values for the run are valid. If the run is invalid (contamination – detection of the band of 280 bp in the Negative Controls), the entire test protocol (sample preparation, amplification and detection) should be repeated. Discard any reagents that may be suspect.
- A sample is considered to be inhibited if no band of 700 bp is observed on agarose gel. Repeat the test.
- The sample is considered to be negative if the single band of 700 bp is observed on agarose gel.
- The sample is considered to be positive for *Mycobacterium Paratuberculosis DNA* if the band of 209 bp is observed on agarose gel.

