DATA SHEET

REF V-9-50R
VER 12.05.10

NEW VERSION

IVD For *in Vitro* Diagnostic Use

CE

EBV 290

Key to symbols used

REF	List Number		Store at 2-8°C
IVD	For *in Vitro* Diagnostic Use		Caution!
LOT	Lot Number	VER	Version
	Expiration Date		Consult instructions for use
CONTROL -	Negative Control	CONTROL +	Positive Control
Σ	Contains reagents		Manufacturer

NAME
EBV 290

INTENDED USE
EBV 290 is an *in vitro* nucleic acid amplification test for qualitative detection of EBV.

PRINCIPLE OF ASSAY
EBV 290 Test is based on three major processes: sample preparation, nucleic acid amplification of DNA using specific EBV primers and detection of the amplified products on agarose gel. EBV 290 kit contains the internal control (human beta-globine gene), which allows to control the presence of cellular material in the sample.

MATERIALS PROVIDED
- PCR-mix-1 55 ready-to-use single-dose test tubes;
- PCR-mix-2, 0,6 ml;
- EBV & Human DNA C+, 0,1 ml;
- Negative Control C-*, 1,2 ml;
- DNA buffer, 0,5 ml;

Contains reagents for 55 tests.
*can be used in the isolation procedure as Negative Control of Extraction.

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, REF K-1-1/B)
- Detection agarose kit

WARNINGS AND PRECAUTIONS
1. Wear disposible gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Do not pipette by mouth. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
3. Do not use a kit after its expiration date.
4. Dispose all specimens and unused reagents according with local regulation.
5. Specimens should be considered potentially infectious and handled in biological cabinet in accordance with Biosafety Level 2 or other appropriate biosafety practices.
6. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
7. Avoid contact of specimens and reagents with the skin, eyes and mucose membranes. If this solutions comes into contact, rinse immediately with water and seek medical advice immediately.
8. Material Safety Data Sheets (MSDS) are available on request.
9. This kit is elaborated for use with "DNA-Sorb-B" extraction kit. It is user's responsibility if kits other than "DNA-Sorb-B" are used to perform this DNA extraction.
10. Use of this product should be limited to personnel trained in the techniques of PCR.
11. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Rilevation Area. Do not return samples, equipment and reagents in the area where you performed previous step.

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STORAGE INSTRUCTIONS
EBV 290 must be stored at 2-8°C.

STABILITY
EBV 290 is stable up to the expiration date indicated on the kit label.

SAMPLE COLLECTION, STORAGE AND TRANSPORT
EBV 290 can analyze DNA extracted with **DNA-Sorb-B** (REF K-1-1/B) from:

- *Whole blood* collected blood in ACD or EDTA tubes;
- *liquor* stored in "Eppendorf" tube;
- *amniotic liquid* stored in "Eppendorf" tube;
- *Sputum* collected in "Eppendorf" tube
- *tissue* homogenized with meccanical homogenizer and dissolved in PBS sterile;

Specimens can be stored at +2-8°C for no longer than 48 hours, or frozen 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
1. Prepare required quantity of tubes **PCR-mix-1**.
2. Pipette **10 µL** of **PCR-mix-2** 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 thermalcycler only when temperature reached 95°C and start the following program:

	Thermocyclers with active temperature adjustment **"GeneAmp PCR System 2400" (Applied Biosystems), "Palm Cycker" (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)**		
Step	t°C	Time	Cycles	t°C	Time	Cycles	t°C	Time	Cycles
0	95°C	Pause		95°C	Pause		95°C	Pause	
1	95°C	5 min	1	95°C	5 min	1	95°C	5 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	4°C	Storage		4°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:

- *EBV* - **500 bp**
- *IC (human β-globine gene)* – **723 bp**

RESULTS INTERPRETATION
Table 2. Results for controls

Control	Which step of test is controlled	Specific bands in the gel **500 bp**	Specific bands in the gel **723 bp**	Interpretation
NCS	DNA isolation	No	No	Valid result
DNA-buffer	Amplification	No	No	Valid result
C+	Amplification	**Yes**	**Yes**	Valid result

The sample is considered to be positive for *EBV DNA* if the band of 500 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 EBV primers and probes. The specificity of the kit EBV 290 was 100%. The potential cross-reactivity of the kit EBV 290 was tested against the group control. It was not observed any cross-reactivity with other pathogens.

Analytical sensitivity
The kit EBV 290 allows to detect EBV 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: LMP