CE

IVD

For Professional Use Only

Cytolysin

nucleic acid extraction kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

Cytolysin nucleic acid extraction kit is intended for the extraction of DNA from whole blood leukocytes.

2. PRINCIPLE OF NUCLEIC ACID EXTRACTION

Cytolysin nucleic acid extraction kit is a reagents kit for rapid and efficient manual extraction of DNA from whole blood leukocytes. Cytolysin solution is intended for cell lysis; it contains protease. Hemolytic solution is intended for erythrocyte lysis.

Extracted DNA may be used for PCR diagnostic tests.

3. CONTENT

Cytolysin nucleic acid extraction kit is produced in 1 form:

Cytolysin nucleic acid extraction kit, **REF** K1-3-100-CE;

Cytolysin nucleic acid extraction kit includes:

Reagent	***Description***	***Volume (ml)***	***Amount***
Hemolytic	colorless clear liquid	100	2 vials
Cytolysin	colorless clear liquid	5.0	2 vials

Cytolysin nucleic acid extraction kit is intended for 100 DNA extractions, including controls.

4. ADDITIONAL REQUIREMENTS

- Disposable powder-free gloves and laboratory coat
- PCR box
- Pipettes (adjustable)
- Sterile pipette tips with aerosol barrier (up to 200 µl and 1,000 µl)
- Sterile pipette tips (up to 200 µl)
- 1.5 ml disposable polypropylene screw-cap microtubes
- Tube racks
- Vortex mixer
- Desktop microcentrifuge with rotor for the reaction tubes (RCF max. 16,000 x g)
- Thermostatic bath or dry block for tubes with controlled temperature and capable of incubating at 25-100 °C
- Vacuum aspirator with flask for removing supernatant
- Refrigerator for 2–8 °C.
- Permanent pen for labeling
- 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 extracted positive material (samples, controls and amplicons) away from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, 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 expiry 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 compliance 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 mucose membranes. If skin, eyes and mucose membranes 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 move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.

6. SAMPLING AND HANDLING

Sampling of biological materials for PCR-analysis, transportation and storage are described in detail in handbook of the manufacture [1]. It is recommended that this handbook is read before starting the work.

Cytolysin nucleic acid extraction kit is recommended for **DNA** extraction from:

- *whole blood*

Collect 2 ml of blood in a tube with 0.1 ml of 6% EDTA. Seal the tube and invert it 3-4 times to ensure adequate mixing. Store the blood specimen at 2-8 ºC for up to 48 hours.

7. WORKING CONDITIONS

Cytolysin nucleic acid extraction kit should be used at 18–25 °C.

8. PROTOCOL

Shake the vial with cytolysin before starting the operations

8.1. DNA Extraction

1. Prepare required quantity of 1.5 ml disposable polypropylene screw-cap microtubes including one tube for Negative Control of Extraction and one for Positive Control of Extraction.
2. Add **1.0 ml** of **hemolytic** to each tube except for controls. Label the test tubes.
3. Transfer **0.25 ml** of **blood sample** per each tube. Tightly seal the tubes and mix on vortex.
4. Incubate the tubes at room temperature for 5 min, vortex, then incubate for 5 min once again.
5. Centrifuge all tubes at 6,000 r/min for 3 min.
6. Carefully remove the supernatant using vacuum aspirator (ensure that the pellet is not disturbed). Use a new tip for each sample.
7. Add **0.5 ml** of **hemolytic** per each tube with the sediment. Mix the tubes on vortex, and incubate at room temperature for 5 min.
8. Centrifuge all tubes at 6,000-8,000 r/min for 3 min. Carefully remove the supernatant using vacuum aspirator (ensure that the pellet is not disturbed) and a new tip for each sample.
9. Repeat washing with the **hemolytic.**
10. Shake the vial with **cytolysin**.
11. Add **0.1 ml** of **cytolysin** into each tube with leukocyte sediment. Immediately re-suspend the cell pellet using tip with aerosol barrier. Tightly screw the tube caps and vortex.
12. Prepare Controls as follows:

12.1 To the tube for Positive Control of Extraction add **0.1 ml of cytolysin** and **10 µl of Positive Control DNA** (provided with the amplification kit).

12.2 To the tube for Negative Control of Extraction add **0.1 ml of cytolysin** and **10 µl of DNA-buffer.**

13. Incubate all tubes at 60 ºC for 30 min. Mix the tubes on vortex every 10 min while incubating (for better dissolving). After that, incubate the tubes at 95 ºC for 20 min.
14. Centrifuge the tubes at 10,000 rpm for 1 min.

The supernatant contains DNA and is ready for PCR amplification.

If using the DNA samples for a diagnostic assay, follow the instructions supplied by the manufacturer.

8.2. Amplification.

Different manufacturers offer PCR amplification kits. We recommend using AmpliSens® PCR amplification kits.

Please carry out the amplification according to the manufacturer instruction.

9. TROUBLESHOOTING

These troubleshooting guides may be helpful in explaining any problem that may arise.

False negatives with extraction product:

- Degradation of the nucleic acid contained in the sample. Use a new sample, store samples appropriately.
- Loss of nucleic acid residue. Carefully draw off the washing solution and try not to remove the nucleic acid residue.
- Degradation of the extracted nucleic acid. Plastic free from DNAses and RNAses should be used.

False positives from extracted product:

- Contamination during sample extraction. It's necessary to open one test tube at a time. Avoid spilling the contents of the test tube, always change tips.
- Contamination of the reagents prepared for the step. It's necessary to repeat the test.
- Contamination of the extraction zone by amplicons. It's necessary to clean surfaces and instruments using aqueous detergents, wash lab coats, replace test tubes and tips in use. Use different laboratory coats in different Amplification areas.

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

10. TRANSPORTATION

Cytolysin nucleic acid extraction kit should be transported at 2–8 ºC.

11. STABILITY AND STORAGE

All components of the of **Cytolysin** nucleic acid extraction kit are to be stored at 2-8 ºC, when not in use. They also must be stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

12. REFERENCES

1. Manual “Sampling, transportation and storage of clinical material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”, Moscow, 2008

13. QUALITY CONTROL

In accordance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485 – certified Quality Management System, each lot of **Cytolysin** nucleic acid extraction kit is tested against predetermined specifications to ensure consistent product quality.

14. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation	EC REP	Authorised representative in the European Community
	Manufacturer		Date of manufacture

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
27.12.10 KM	Cover page	The phrase “For Professional Use Only” was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
30.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”