



For *in Vitro* Diagnostic Use

# Citolizin

## Nucleic acid Extraction kit

### Instruction Manual



#### TABLE OF CONTENTS

1. INTENDED USE.....	2
2. PRINCIPLE OF NUCLEIC ACID EXTRACTION.....	2
3. CONTENT.....	2
4. ADDITIONAL REQUIREMENTS.....	2
5. GENERAL PRECAUTIONS.....	2
6. SAMPLING AND HANDLING.....	2
7. PROTOCOL.....	3
8. TROUBLESHOOTING.....	3
9. STABILITY AND STORAGE.....	4
10. REFERENCES.....	4
11. QUALITY CONTROL.....	4
12. EXPLANATION OF SYMBOLS.....	4



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#### 1. INTENDED USE.

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

#### 2. PRINCIPLE OF NUCLEIC ACID EXTRACTION.

**Citolizin** 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.

**Citolizin** nucleic acid extraction kit is produced in 1 form:

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

**Citolizin** 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

**Citolizin** 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 between 25°C and 100 °C
- Vacuum aspirator with flask for removing supernatant
- Refrigerator for temperature between 2 and 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.

**Citolizin** 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 from 2 to 8 °C for up to 48 hours.

## 7. PROTOCOL.



Shake the vial with cytolysin before starting the operations

### 7.1. DNA Isolation

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 °C for 30 min. Mix the tubes on vortex every 10 min while incubating (for better dissolving). After that, incubate the tubes at 95 °C for 20 min.

14. Centrifuge the tubes at 10,000 r/min 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.

### 7.2. Amplification.

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



Please carry out the amplification according to the manufacturer instruction.

## 8. 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.

## 9. STABILITY AND STORAGE.

All components of the of **Citolizin** nucleic acid extraction kit are to be stored between 2 °C and 8 °C, when not in use. They also must be stable until the expiry date stated on the label.

## 10. REFERENCES.

1. Manual "Sampling, transportation and storage of clinical material for PCR diagnostics", developed by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008

## 11. QUALITY CONTROL.

In accordance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 – certified Total Quality Management System, each lot of **Citolizin** nucleic acid extraction kit is tested against predetermined specifications to ensure consistent product quality.

## 12. EXPLANATION OF SYMBOLS.



Manufacturer



Use by



For *in Vitro* Diagnostic Use



Catalogue number



Contains sufficient for <n> tests



Consult instructions for use



For working with Rotor-Gene™ 3000/6000



Positive control



Temperature limitation



Batch code



Version



Internal Control complex



Authorized representative in the European Community.



Caution, consult accompanying documents



For working with iQ5, iQ iCycler



Negative control