DATA SHEET

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CE

Bacillus Anthrax PCR Kit
Cat. No.: ZD-0073-03

For use with Conventional PCR Instrument or Real time PCR Instrument

For in vitro Diagnostic use only
User Manual

1. Intended Use
Bacillus Anthrax real time PCR kit is used for the detection of Bacillus anthrax in swab, biopsy or serum sample by using conventional PCR instruments or real time PCR instruments.

2. Principle of PCR
PCR is based on the enzymatic amplification of a fragment of DNA that is flanked by two 'primers', short oligonucleotides that hybridize to the opposite strands of the target sequence and then prime synthesis of the complementary DNA sequence by DNA polymerase (an enzyme). The chain reaction is a three-step process, denaturation, annealing, and extension, that is repeated in several cycles. At each stage of the process, the number of copies is doubled from two to four, to eight, and so on. The reactions are controlled by changing the temperature using a special heat-stable Taq polymerase.

3. Product Description
Bacillus Anthrax are the etiological agents of brucellosis, a zoonotic disease endemic in many areas of the world, characterised by chronic infections in animals leading to abortion and infertility, and a systemic, febrile illness in humans. Human infection frequently occurs via direct contact with tissues and fluids from infected animals, but can also be contracted by consumption of contaminated foods or by inhalation. The product contains a reaction Mix for the specific amplification of Anthrax Bacillus DNA. A thermo stable DNA polymerase is used to amplify the specific gene fragments by means of polymerase chain reaction.

4. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns
1	DNA Extraction Buffer	2 vials, 1.5ml
2	Anthrax Bacillus Reaction Mix	1 vial, 950µl
3	PCR Enzyme Mix	1 vial, 12µl
4	Molecular Grade Water	1 vial, 400µl
5	DNA Marker	1 vial, 40µl

5. Storage
- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as this may reduce the assay sensitivity.
- Cool all reagents during the working steps.
- Reaction mix should be stored in the dark.

6. Additionally Required Materials and Devices
- Biological cabinet
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Vortex mixer
- Cryo-container
- Pipets (0.5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and freezer
- Tube racks
- Tape
- Agarose
- Microwave
- Ethidium bromide
- Agarose Gel Electrophoresis apparatus

7. Warnings and Precaution
Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, and smoke in laboratory.
- Avoid aerosols

8. Sample Collection, Storage and transport
- Collect samples in sterile tubes;
- Specimens can be extracted immediately or frozen at -20°C to -80°C.

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• Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. Procedure

9.1 DNA-Extraction

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use.

9.1.1 Swab or biopsy sample

1) Wash the sample in 0.5ml normal saline and vortex vigorously. Centrifuge at 13000rpm for 2 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 100µl DNA extraction buffer to the tube, closed the tube then vortex for 10 seconds.
3) Incubation the tube for 10 minutes at 100°C.
4) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the extracted DNA and can be used for the template of the PCR.

9.1.2 Serum sample

1) Pipet 50µl sample to a new 0.5ml tube, add 50µl DNA extraction buffer, closed the tube then vortex for 10 seconds.
2) Incubation the tube for 10 minutes at 100°C.
3) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the extracted DNA and can be used for the template of the PCR.

Attention:

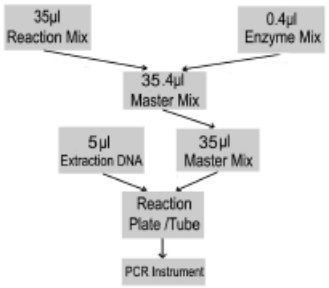
A. During the incubation, make sure the tube is not open, for the vapor will volatilize into the air and may cause contamination if the sample is positive.

B. The extraction sample should be used in 3 hours or stored at -20°C for one month.

C. DNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For DNA extraction, please comply with manufacturer's instructions.

9.2 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



1) Depending upon the number of sample(n) the following pipetting scheme can be followed. (For reasons of unprecise pipetting, always add an extra virtual sample.)

Reaction Volume	Master Mix Volume
35µl Reaction Mix	35µl × (n+1)
0.4µl Enzyme Mix	0.4µl × (n+1)

Mix completely then spin down briefly in a centrifuge.

2) Pipet **35 µl** Master Mix with micropipets of sterile filter tips to each PCR reaction plate/tubes. Separately add **5µl** DNA sample template, molecular grade water to different plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

94°C for 2 min, 1 cycle;
93°C for 15 sec, 55°C for 30sec, 72°C for 30sec, 35 cycles.

9.3 Agarose Gel Electrophoresis

1) Mix the samples of DNA with 0.20 volume of the desired 6x gel-loading buffer.
2) Slowly load the sample mixture into the slots of the submerged gel using a disposable micropipette, an automatic micropipettor, or a drawn-out Pasteur pipette or glass capillary tube. Load DNA Marker into slots on both the right and left sides of the gel.
3) Close the lid of the gel tank and attach the electrical leads so that the DNA will migrate toward the positive anode (red lead). Apply a voltage of 1-5 V/cm (measured as the distance between the positive and negative electrodes). If the leads have been attached correctly, bubbles should be generated at the anode and cathode (due to electrolysis), and within a few minutes, the bromophenol blue should migrate from the wells into the body of the gel. Run the gel until the bromophenol blue and xylene cyanol FF have migrated an appropriate distance through the gel.
4) When the DNA samples or dyes have migrated a sufficient distance through the gel, turn off the electric current and remove the leads and lid from the gel tank. Then, examine the gel by UV light and photograph the gel.

10. Data Analysis and Interpretation

The following results are possible:

1) Through agarose gel electrophoresis, a expected **175 bp** PCR product is observed from a sample. **The result is positive: The sample contains Anthrax Bacillus DNA.**
2) Through agarose gel electrophoresis, no expected **175 bp** PCR product is observed from a sample. **The result is negative: The sample does not contain Anthrax Bacillus DNA.**