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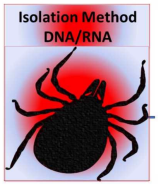
A new DNA / RNA isolation protocol from a single tick for molecular analysis of various pathogens such as Borrelia, Babesia, Anaplasma, TBE, Alongshan virus etc.



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Sudhir Bhatia[1], Gudrun Baersch[1]

[1]Genekam Biotechnology AG

Sudhir Bhatia
Genekam Biotechnology AG

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We use this protocol and it's working

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Disclaimer

Genekam Biotechnology AG
Duissernstrasse 65a
47058 Duisburg
Germany

Abstract

We describe here a rare protocol for the isolation of nucleic acid from a single tick sample:

Ticks carry a variety of deadly pathogens, e.g. *Borrelia*, *Babesia*, *Anaplasma*, Alongshan virus and tick-borne encephalitis virus. It is therefore crucial to detect the presence of these pathogens. Such detection is possible using molecular analysis methods such as conventional and real-time PCR.

It is also important to pick up the tick that has bitten someone instead of throwing it away. This tick can be examined in the laboratory for the presence of pathogens, making it easier to diagnose and provide appropriate treatment.

There is no strategy for shipping the tick or temperature information in the literature. Many studies describe cumbersome methods such as storage and shipping in liquid nitrogen. In this protocol, we demonstrate how easy it is to transport a tick at outside temperature from one location to another using a postal letter or courier parcel.

Pooled samples of 8-10 are used in most studies. In contrast, we use an inexpensive and simple method to isolate nucleic acid from a single tick sample. We successfully crushed the tick in buffer using a mortar and pestle from the grocery store. The nucleic acid can be extracted from this. To our amazement, in many cases we obtained up to 800 ng/ul of nucleic acid per tick as measured with spectrometer, and we have performed many pathogen specific tests with this extracted nucleic acid over the past 15 years for more than 250 tick samples.

Our approach, based on individual tick samples, will provide new opportunities for research laboratories around the world to determine the exact presence of pathogens. It is also highly recommended to show learners the latest applications in medicine and biotechnology. Read the publications provided in this protocol too.

Image Attribution

DNA/RNA isolation tick

Guidelines

Read the manual before use.
Check the contents of the kit.

Precautions:

- The kit is only for in vitro use.
- The kit may only be used by trained persons.
- The user must read the manual carefully.
- It should not be used after expiry date.
- The user should work very cleanly when isolating.
- Decontaminate the instruments regularly (once a week).
- The user should wear protective gloves and laboratory clothing.

Materials

Genekam Isolation Kits:
-SB0079 - Genekam Universal RNA-Isolation Kit (INT)
-UDI-DI: 04262420430799 - CE
-SB0001 - Genekam Universal DNA-Isolation Kit (INT)
-UDI-DI: 04262420430706 - CE
-SB0179 - Genekam Universal DNA/RNA-Isolation Kit (INT)
-UDI-DI: 04262420430829 - CE

Contents:
-Tube A (lysis buffer 1)
-Tube G (lysis buffer 2)
-Tube K (proteinase K) to be stored at 4°C
-Tube B (washing buffer 1)
-Tube C (washing buffer 2)
-Tube E (elution buffer)
-Mini columns
-Collection tubes for mini column (2ml with round bottom)
-Collection tubes for mini column (1.5ml with conical bottom) for elution

Chemicals and equipment needed:
-Molecular Ethanol- The use of molecular Ethanol produces the best quality results!
-Pipettor and Pipette tips
-Heat block
-Centrifuge
-Vortexer
-Burner
-Mortal and pestle

Safety warnings

-Keep the kit away from sun light.
-If the package and the bottles are damaged don´t use the kit.
-Read the material safety data sheet.

Before start

-Read the protocol before start.
-Make the heat block on and adjust the temperature as given in the protocol.
-Elution buffer should be pre warmed.

1 The tick can be sent in a letter, as shown in the picture, or in a small plastic tube (tightly closed). If the tick is still alive, care must be taken to ensure that it does not escape. It is therefore important to look at the tick with a magnifying lens, as some ticks are very small and transparent.

2 Put 100µl of Tube A into the pestle and throw in the tick or add the tick. Now crush the tick with the mortar. This liquid with the crushed tick should be used as input for the isolation of the tick. 3m

Mortar and Pestle

Hint: Sterilization of mortar and pestle: They must be washed thoroughly with tap water and then rinsed twice with double distilled water. Leave them to dry. The surface of the mortar and pestle should be treated gently for a few seconds with the flame of a gas burner without damaging the surface before use to destroy any DNA on the surface. In this way, the mortar and pestle can be reused. We have been working very successfully with this method for 15 years.

3 Incubate for 20-30 minutes at 56°C. Add 400µl of Tube G. Incubate for 5 minutes at 70°C. 30m

4 Add 400µl of molecular ethanol and shake with the vortexer.

5 Take a mini-column in a collection tube and add 600µl of the solution prepared above to this mini-column.

6 Centrifuge the solution for one minute at 11000rpm. Discard the filtrated fluid. 1m

7 Add the remaining liquid to this mini column and repeat step 5 for centrifugation. Discard the filtrated fluid. 1m

8 Now add 500µl of Tube B to the mini column. Repeat step 5 for the centrifugation and discard the filtrated fluid. 1m

9 Add 500µl of Tube C to the mini column. Repeat step 5 for the centrifugation and discard the filtrated fluid. 1m

10 Add 200µl of Tube C to the mini column. Repeat the centrifugation for 3 minutes at 13000rpm and discard the filtrated fluid. 3m

11 Centrifuge the mini column for 1 minute at 13000rpm to dry the matrix. Dispose of the used collection tube. 1m

12 Now place the mini column (filter part) in a new 1.5 ml collection tube.

13 Add 100µl of the Tube E preheated to 70°C to the mini column.

14 Allow the sample to stand at room temperature for two minutes. 2m

15 Centrifuge for one minute at 13000rpm. 1m

16 Now the user has liquid in the collection tube. This is isolated nucleic acid. This can be used to perform various tests. The concentration of isolated samples may be measured with a spectrometer. The nucleic acid should be stored at -20°C for long-term use.

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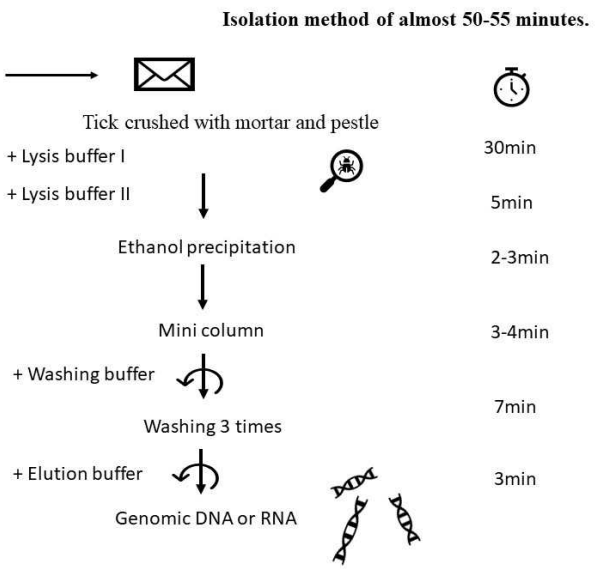

Scheme for nucleic acid isolation from the tick

Protocol references

Baersch G, Bhatia S. Relationship between the spectrometric values of DNA, RNA, and the PCR presence of a pathogen in single tick samples. Biotechnologia Acta. 2024;17(1). https;//doi.org/10.15407/biotech17.01.062