analytikjena | Biometra

Life Science unlimited

Manual

innuAMP Tick DNA Test

Order No.:

845-IA-2006010	10 reactions
845-IA-2006025	25 reactions
845-IA-2006050	50 reactions

Publication No.: HB_IA-2006_e_120618

This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

Expression and further use permitted with indication of source.
© Copyright 2012, Analytik Jena AG, AJ Innuscreen GmbH

Manufacturer:
AJ Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin
Made in Germany!

Distribution/Publisher:
Analytik Jena AG
Konrad-Zuse-Straße 1
07745 Jena/Germany

Phone +49 (0) 36 41 / 77-94 00
Fax +49 (0) 36 41 / 77-76 77 76
www.bio.analytik-jena.com
lifescience@analytik-jena.com

Inhalt

1 Introduction and description

The innuAMP Tick DNA Test was designed to determine tick specific DNA and thus to verify the isolated DNA from ticks via a PCR amplification especially referring to further diagnostic determinations for the detection of bacterial pathogens.
A negative amplification result should be used for the critical appraisal of the other PCR fragments, which were amplified using the same DNA template. The usage oft he innuAMP Tick DNA Test, hence avoids false negative responds of further microbial DNA determinations.
The amplification protocols for a tick specific mitochondrial gene are optimally adapted to the unique rapidPCR technology using Low Profile Rapid (LPR) blocks or Standard Profile Rapid (SPR) blocks (Analytik Jena AG), as well as optimized to the usage of standard PCR thermal cyclers, e.g. FlexCycler (Analytik Jena AG).
Due to the optimization oft he whole test system, the DNA of all species of the Ixodidae family (hard ticks) could be determined.
The amplification product (150 bp) and a DNA ladder (e.g. Fermentas; 1kb DNA ladder) are added to a 2 % TAE gel after the PCR is finished.
A visible amplification fragment, in comparison to one negative control, is manifesting a successful DNA extraction.

2 Performance assessment, spectrum of application and specificity

This test was used to determine the occurrence of DNA from about 100 ticks for amplification by PCR.

- Dermacentor reticulatus
- Ixodes hexagonus
- Haemaphysalis concinna
- Ixodes ricinus

The following table gives an overview of the results, which were determined in this study.

Tick species	**Number**	
	Complete	**Result** (determination of tick specific DNA)
Dermacentor reticulatus	20	20
Ixodes hexagonus	1	1
Haemaphysalis concinna	5	5
Ixodes ricinus	74	74
Complete	**100**	**100**

3 Kit components, storage and stability

Important!
The master mix has to be prepared freshly before each application. Storage of a ready-to-use master mix could lead to false positive.

Note!
Only for the usage of 36, 96 well microplates LP, 8 well strips LP or 8 well strips 0.2 ml.

Component	Content per reactions			Storage
	10	25	50	
8 well strip LP with sealing foil	3	5	10	Room temperature
8 well strip 0.2 ml with lid	2	4	7	Room temperature
Tick DNA positive control	7.5 µl	15 µl	25 µl	- 20 °C
Primer 1 Z	25 µl	60 µl	100 µl	- 20 °C
Primer 2 Z	25 µl	60 µl	100 µl	- 20 °C
dNTP mix	10 µl	20 µl	40 µl	- 20 °C
10x SpeedAmp PCR Buffer	50 µl	100 µl	200 µl	- 20 °C
10x PCR Buffer	50 µl	100 µl	200 µl	- 20 °C
PCR-grade H_20	250 µl	500 µl	1000 µl	- 20 °C
innuTaq HOT DNA Polymerase	10 µl	15 µl	20 µl	- 20 °C work on ice

4 Necessary laboratory equipment and und additives

- SpeedMill (Analytik Jena AG) or other commercial available homogenizers on the basis of beads
- rapidPCR thermal cycler with a Low Profile Rapid (LPR) block / Standard Profile Rapid (SPR) block (Analytik Jena AG) or a standard PCR thermal cycler with heated lid and 0.2 ml wells (e.g. FlexCycler, Analytik Jena AG)
- Microcentrifuge
- Vortexer
- Variable pipettes for 10 µl, 100 µl and 1.000 µl (use separate pipettes for extraction, amplification and detection)
- Sterile pipette tips with protection against contamination (filter tips)
- DNA ladder for detection of a 150 bp DNA fragment (e.g. innuSTAR 100 bp DNA ladder, Analytik Jena)
- Agarose
- Gel-electrophoresis
- Gel documentation system or transilluminator

5 Remarks and safety precautions

All reagents in this kit only have to be used for the intention mentioned inside the user manual. The application may only be exercised by authorized personal.

During the operation, the described protocol has to be followed strictly. Furthermore the regularities to operate quality controls within medical laboratories have to be considered.

The reagents should be stored inside the original vessels at the mentioned temperatures. Single components of different charges and consumables may not be exchanged. The mentioned expiry dates have to be considered.

The material to be determined has to be categorized as potential infectious. The accordant precautions have to be noticed.

For the exposure to the kit reagents and the sample material, the accordant regulations to prevent accidents for the medical service have to be observed. Particularly the following precautions have to be considered:

- Don't eat, drink or smoke!
- Always wear protective clothing and gloves!

The reagent vessels could be disposed with the normal laboratory waste.

6 Performance of the test

Important notes!

- Do not exchange the components of different kits or kit charges
- Open and close the vessels of single components always separately
- Change contaminated gloves immediately
- Spatial separation of the amplification and detection area
- Follow the below mentioned order of the laboratory operational procedure:

    1. Sample preparation (nucleic acid extraction)
    2. Amplification
    3. Detection

- Do not open PCR plastics, which contain amplified samples in the area of sample preparation (DNA isolation) or preparation of amplification
- Amplified samples and controls are potential sources of contamination
- Use separate pipettes with sterile filter tips for the preparation of the PCR reaction master mixes
- Open the reaction vessels carefully to avoid the generation of aerosols

6.1 Isolation of nucleic acids

The isolation of the nucleic acids has to be done using the blackPREP Tick DNA Kit or the blackPREP Tick DNA/RNA Kit. The protocols inside the accordant user manual have to be followed exactly.

Note: The operation of the test was optimized by using nucleic acids, which were isolated by the above mentioned extraction kits. Alternatively, also nucleic acids, which were isolated by other methods could be used.

6.2 PCR amplification

Note

For usage of 36, 96 well microplates LP, 8 well strips LP or 8 well strips 0.2 ml

The performance of the amplification and the hybridization of the PCR product could be done either using a rapidPCR thermal cycler, as well as using a standard PCR thermal cycler (including a heated lid). The thermal cycler also needs a sample protection system (SPS) that cools samples to the set temperature (105ºC – 120ºC) while the lid is heating in order to prevent primer/probe dimer formation, non-specific annealing and early elongation.

6.2.1 Initial steps

1. Divide the DNA eluates and controls to the accordant PCR plastic

	8 Well Strip LP	8 Well Strip 0,2 ml
Sample (extracted DNA)	1.5 µl	2.5 µl
Positive (positive control)	1.5 µl	2.5 µl
Negative (PCR-grade H_2O)	1.5 µl	2.5 µl

2. The prepared plastic has to be stored on the cooling block until the amplification is started

6.2.2 Preparation of the PCR reaction mix

> **Important!**
> The master mix has to be prepared freshly before each application. Storage of a ready-to-use master mix could lead to false positive results.

1. Thaw all reagents, vortex, spin down and store the components on ice during the preparation
2. The preparation of the master mix for one sample is described in the following table. The preparation of the master mix has to be done for the number of used samples (including positive and negative controls)

Mastermix	**rapidPCR**		**standard PCR**
Plastic	8 well strip LP (20 µl)	8 well strip (0.2 ml)	8 well strip (0.2 ml)
10x SpeedAmp PCR Buffer	1.5 µl	2.5 µl	-
10x PCR Buffer	-	-	2.5 µl
Primer 1 Z	1.0 µl	1.5 µl	1.5 µl
Primer 2 Z	1.0 µl	1.5 µl	1.5 µl
dNTP Mix	0.3 µl	0.5 µl	0.5 µl
innuTaq Hot DNA Polymerase	0.15 µl	0.25 µl	0.25 µl
PCR-grade H_2O	8.55 µl	14.75 µl	14.75 µl
Final PCR volume	15 µl/reaction	25 µl/ reaction	25 µl/ reaction

3. The master mix has to be added to the wells, which still contain the pre-pipetted DNA samples (positive and negative controls respectively) as described in the following

	rapidPCR		standard PCR
Plastic	8 well strip LP (20 µl)	8 well strip (0.2 ml)	8 well strip (0.2 ml)
Master mix	13.5 µl	22.5 µl	22.5 µl
Finale PCR volume	15 µl/reaction	25 µl/ reaction	25 µl/ reaction

4. Seal the PCR plastic with the accordant foil (PP), put it into the thermal cycler and close the lid
5. Start the PCR time and temperature protocol

6.2.3 Amplification

The PCR protocol contains the following steps:

Step 1: Initial PCR activation step

Step 2: Amplification of the tick specific DNA fragment.

> **!** **Attention!**
> The following PCR protocols are adapted to the accordant PCR thermal cycler.

rapidPCR thermal cycler with LPR or SPR block:

Amplification

Step	Cycles	Profile	Temperature	Holding time	Ramp rate
1	1	Initial denaturation	95 °C	120 sec	max
2	37	Denaturation	95 °C	4 sec	max
		Annealing	50 °C	4 sec	max
		Elongation	72 °C	20 sec	max

Standby: 18 °C

Time: approx. 32 min

Standard PCR thermal cycler:

Amplification

Step	Cycle	Profile	Temperature	Holding time	Ramp rate
1	1	Initial denaturation	95 °C	120 sec	max
2	37	Denaturation	95 °C	30 sec	max
		Annealing	50 °C	30 sec	max
		Elongation	72 °C	60 sec	max

Standby: 18 °C **Time:** depending on thermal cycler

7 Determination and analysis

A. Preparation of a 2 % agarose gel

Prepare a 2 % TAE agarose gel during the PCR reaction is running.

1. Weight **2 g agarose** and add **100 ml 1x TAE buffer**; heat it until the agarose is dissolved and cool it to approx. 60 °C
2. Add **1 µl ethidium bromide** (stock solution: 10 mg/ml) per 100 ml gel solution
3. Pour the gel using a comb
4. Assemble the gel in a tank and add 1x TAE running buffer until the gel is covered by a few millimeters
5. Remove the comb.

B. Loading of the gel

1. After the PCR run is finished take the accordant PCR plastic out of the rapidPCR cycler and remove the sealing foil
2. Add **Loading dye** (**1 µl/10µl sample**) to each sample (e.g. 10 x Orange G)
3. Add the sample and a size marker (e.g. innuSTAR 100 bp DNA ladder, Analytik Jena) onto the 2 % agarose gel
4. Start the electrophoresis (running time: approx. 20 min at 100 mA; until the loading dye is moved approx. 3 – 4 cm into the gel)

C. Analysis

Put the gel to a transilluminator (gel documentation system).

In case of a successful DNA extraction from a tick, a 150 bp fragment have to be visible. Thereby the negative control has to be empty from any band beside primer dimers.

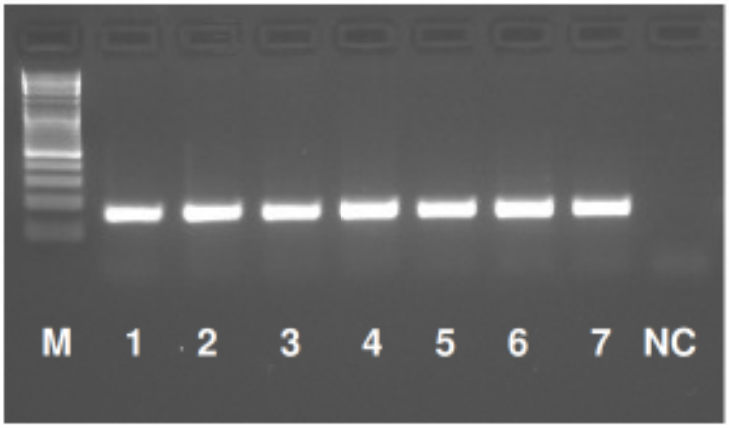

Fig. A: Example of an amplification

Lane M: Marker (DNA ladder)
Lane 1 - 7: Positive samples
Lane NC: Negative control

1. A 150 bp fragment is visible on the gel. The negative control doesn't show a band.	The sample is **positive** and can be used for further determinations.
2. No amplification product is visible on the gel The negative control is empty.	The sample is **negative**, resultant from an unsuccessful DNA extraction or inhibitors of the PCR reaction in the DNA sample.
3. A 150 bp fragment is visible on the gel. The negative control is positive	The sample can not be analyzed positive. The chemicals are contaminated with the PCR product. The experiment should be repeated using a new charge of chemicals.

engl. 06/12 – Analytik Jena AG, Jena

Analytik Jena AG
Life Science
Konrad-Zuse-Strasse 1
07745 Jena / Germany

Phone +49 (0) 36 41 77-94 00
Fax +49 (0) 36 41 77-76 77 76

lifescience@analytik-jena.com
www.bio.analytik-jena.com