© 2011 Genekam Biotechnology AG
MADE IN GERMANY

Lot-No.

MANUAL – one step

Ref. K291

EXPIRY DATE: 1 year

STORE AT -20°C

100 Tests (Ready to use kit)

BARTONELLA GENUS

-Only for in vitro use-
-Only for veterinary use-
-To be used by a technical person-

Principle and use

This amplification kit has been manufactured by *Genekam Biotechnology AG*, Germany to detect *Bartonella genus* (in one step).

This kit needs isolated DNA. Kindly use good methods to isolate the DNA.

Samples: samples from skin lesions, body tissues, nasal swabs, body fluids.

IMPORTANT: we added cotton or sponge in the lid of container of the kit to avoid damage during transportation. Please remove this cotton or sponge from the lid of each container before storage.

Composition:

It contains the following:

• Tube A (2 tubes)
• Tube B (2 tubes)
• Positive (+Ve) Control (tube D1) (1 tube): not included. It can be made from a positive samples.
• Negative (-Ve) Control (tube D2) (1 tube)
• Marker (tube E) : 100bp (max 1000 bp) (1 tube): 100, 150, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 bp
• Dye (tube F) (1 tube)

Please check them before you start.

Equipment needed:

• PCR thermocycler
• Laboratory centrifuge
• UV plateform
• UV safety goggles
• microtubes (0.2ml)
• Pipette-tips with and without filter (20µl, 5µl & 1µl)
• Pipettes (quality pipettes)
• Gel Agarose chamber
• Power supply
• Paper
• Pen
• Agarose (good quality)
• Staining (Ethium Bromide)
• TAE buffer 1x
• Ice
• Vortexer

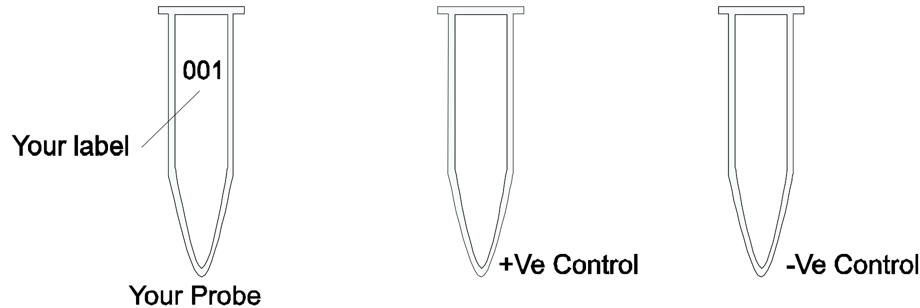
Procedure:

After your DNA isolation is completed. (Kindly use good quality isolation method).

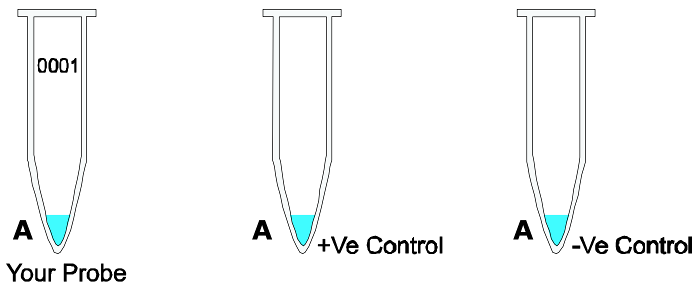
STEP A

1. Kindly thaw **one tube** each of A, B, D1, D2, E and F. After thawing, kindly put the tubes at 4°C (as it is better). If the kit is not in use, store them at -20°C.

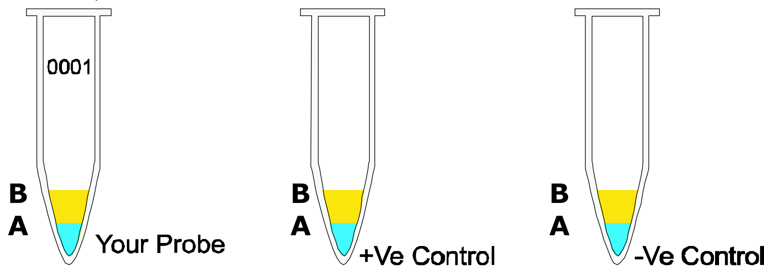
2. Mark your microtubes with a sample number and with +Ve Control and –Ve Control.



3. Thaw tube A. Add 8µl of tube A to each tube.

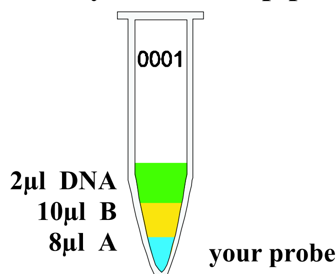


4. Add 10µl of B to each microtube. Avoid to touch the wall of the microtubes.



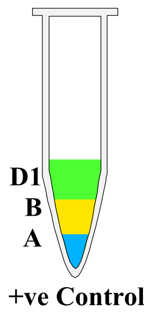
5. **TIP: you can calculate the total requirement of chemicals needed . You need 8µl A + 10µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 80µl of A + 100µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save time and hardware.**

6. Add 2µl of your DNA template (DNA isolated from samples) with pipette tip with filter to each microtube according to your label except +Ve and –Ve (Avoid touching the wall). **Use everytime a new pipette tip** (for each sample)! Mix it thoroughly.

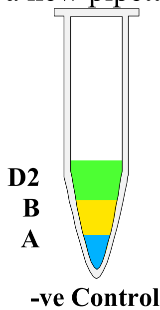


7. Use new pipette tip with filter. Add 2µl of +Ve (tube D1) to +Ve Control (avoid to touch the wall). Use a new pipette tip. Mix it. The positive control can be made from the user. If it is

not available, please run the assay without this.

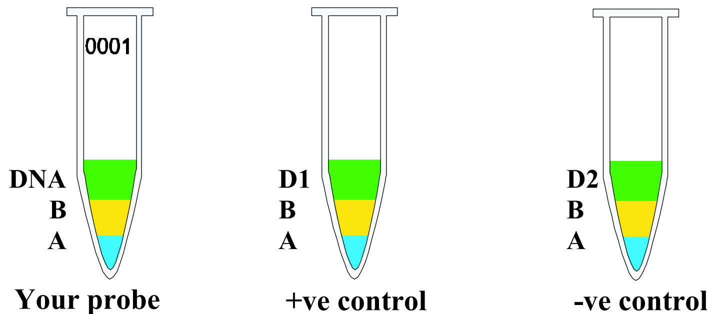


8. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control . Mix it.



9. Centrifuge all tubes for 20 sec. for 8000 rpm (this is not necessary but it is better).

10. Run the programm of your thermocycler as followings:
Kindly check whether you have added everything correctly as the level of the volume of each microtube must be almost the same.



Now program your PCR machine as follows.

1. 300 seconds at 95°C

2. A. 45 seconds at 95°C
   B. 45 seconds at 54°C
   C. 45 seconds at 72°C
   } 45 cycles

3. 600 seconds at 72°C

Before you start the PCR program, kindly check whether tubes are closed properly. **Microtubes must be in contact with metal block** (very important!). There should be no air or lose contact with metal block of thermocycler. Run PCR now.

10. After step 9 is finished take out the microtubes.

To see *Bartonella genus*, you can go directly to step gel electrophoresis (STEP B).

STEP B

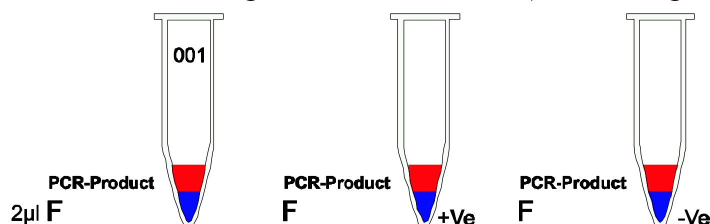
1. Prepare the gel Agarose **2%** in TAE (1x) buffer.

© 2011 Genekam Biotechnology AG
MADE IN GERMANY

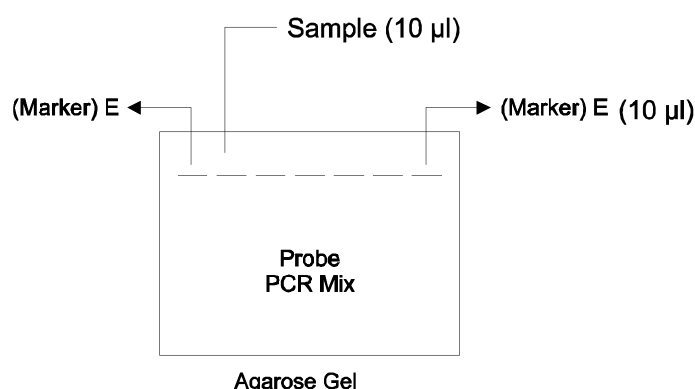
2. Let the gel dry and add this TAE (1x) buffer in gel chamber.

3. Take the tube E (marker). Make ready to use for gel electrophoresis.

4. After the PCR step is finished, now you can prepare for gel Agarose electrophoresis. Take 2µl of dye (tube F) and add to each microtube (with the same number as your PCR microtubes including +Ve & -Ve Controls) containing PCR product.



5. Add 10µl of Marker (Tube E: 100bp) to first and last lane of electrophoresis. (Kindly make lane plan on paper according to your probes in order to identify later and see the results).



6. Add 10µl of mix of step 4 to each lane of gel Agarose (between first and last lane). Change the pipette tip for each lane.

7. Run the gel for **45min**. at **100 Volt**.

8. Make staining solution ready.

9. Put the gel for 5-15 minutes staining solution (0.5µg/ml).

10. View the gel under UV light. UV light is dangerous for your eyes. Use UV goggles.

11. You must find the bands in +Ve Controls and no bands in –Ve controls. (**different according to species** in +Ve Control Bartonella genus and positive samples, please see on extra page for sizes of bands).

Recommendation: Genesequencing is strongly recommended to reconfirm the positive results.

If you should find any mistakes, please let us know. Thank you.

Suggestion:
This manual has been written specifically for beginners, hence persons with experience in PCR must use their experience to keep each step as small as possible e.g. you should calculate the amount of the needed chemicals, before starting with testing.

Last update: 17-10-2011
v1.0

Genekam Biotechnology AG
Dammstr. 31-33
47119 Duisburg
Germany
Tel. (+49) 203 / 555858-31,-32,-33
Fax (+49) 203 / 35 82 99
anfrage@genekam.de
http://www.genekam.de

© 2011 Genekam Biotechnology AG

© 2011 Genekam Biotechnology AG
MADE IN GERMANY

Size of the bands

Bartonella alsatica	598bp
Bartonella clarridgeiae	636bp
Bartonella elizabethae	717bp
Bartonella henselae	**684bp**
Bartonella quintata	564bp
Bartonella vinsonii	704bp
Bartonella berkhoffii	453bp
Bartonella bovis	453bp
Bartonella doshiae	550bp
Bartonella grahamii	658bp
Bartonella koehlerae	621bp
Bartonella taylorii	614bp