Lot-No.

MANUAL

Ref. K722

100 Tests (Ready to use kit)

Expiry date: 1 year

STORE AT -20°C

LASSA VIRUS

-Only for in vitro use-
-human, only for research use-
-To be used by a technical person-

Principle and use:

This amplification kit has been manufactured by *Genekam Biotechnology AG*, Germany to detect Lassa virus.

This kit needs RNA which can be isolated from blood, plasma, serum, cell cultures, rats, rodents and any body fluid. Kindly use good methods to isolate the RNA. Kindly take common safety laboratory precautions during working.

Please use gloves during work. Proceed clean and carefully otherwise you may cause contamination problems. Do not touch other objects like pens, chairs etc. during Part 1.

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:

- RNA kit (HX, PF, NTP, RI, RET, DH)
- Tube A (2 tubes)
- Tube B (2 tubes)
- positive (+ve) control (D1): **to be stored at -20°C** (1 tube):
  To be prepared through user. To prepare this, one can use positive material (RNA only). If you do not have this, please run the assay without this.
- Negative (-Ve) Control (tube D2) (1 tube)
- Marker (tube E) (1 tube) (max 1000 bp): 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 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 and pen
- Agarose (good quality)
- Staining (Ethium Bromide)
- TAE buffer 1x
- Ice

• Vortexer

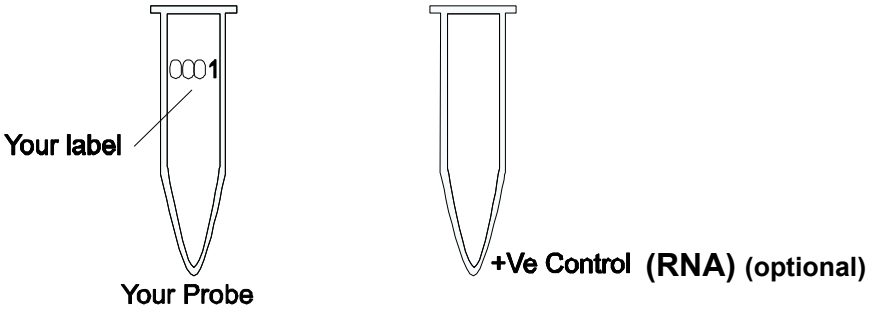
Procedure:

PART 1: Conversion of RNA into cDNA. This part should be done with our RNA.

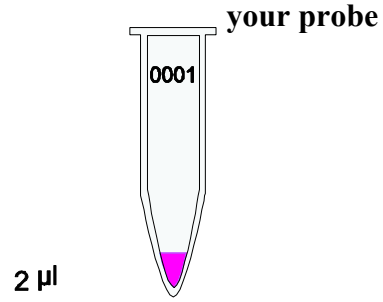
ONCE AGAIN:
VERY IMPORTANT ! PLEASE USE GLOVES ! DON'T TOUCH ANY OTHER OBJECTS, OTHERWISE THERE MAY BE RNASE CONTERMINATION DURING THIS PART.

STEP A

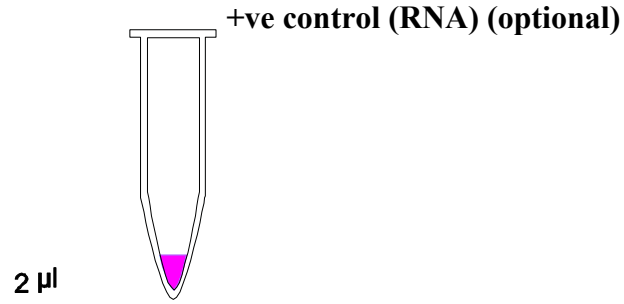
1. Mark your microtubes with a sample number and one with +Ve Control. (RNA), which is optional . It can be made through you.



2. Add 2µl of your isolated RNA from your samples. This may vary e.g. 2.5µl or 3µl.



3. Add 2µl of RNA as positive control to +ve control tube. **This is optional, hence it can be made through you.**

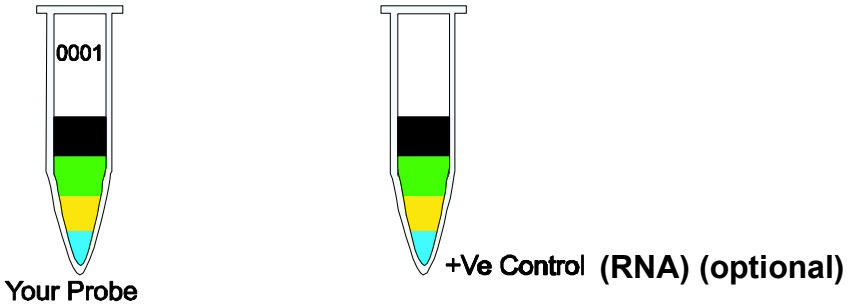


4. Add 1µl of HX (primer) and 9µl of DH (water) to each tube.

4b. Centrifuge for a while (10-15 seconds) and incubate at 70°C for 5 minutes. Afterwards cool it down to 4°C (this can be done in the thermocycler).

TIP: you can calculate your need for HX and DH e.g. you want to run 10 reactions, you need 10µl of HX and 90µl of DH. Mix together and take out 10µl for each tube.

5. Add: 4µl of PF (buffer).
1µl of RI (Inhibitor)
2µl of NTP (nucleotide)
Total: 7µl in tube

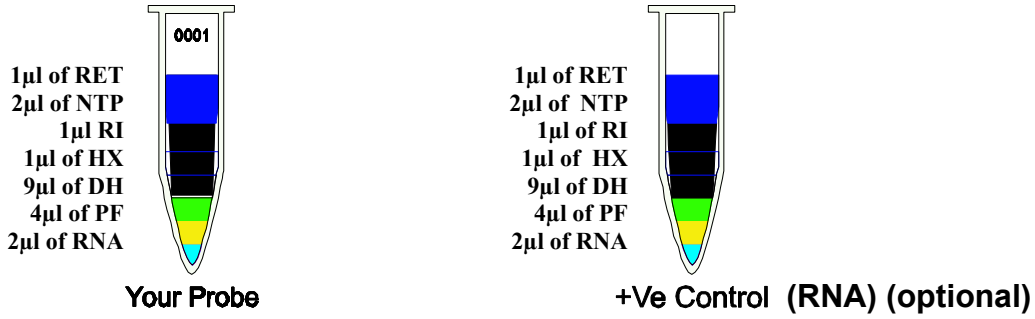


Tip: you can calculate your need for chemicals and mix them together. E.g. for 10 reactions you need 40µl of PF, 10µl of RI and 20µl of NTP = 70µl. After that you can add 7µl to each tube.

6. Run at 25°C for 5 minutes.

7. Add 1µl of RET (reverse transcriptase) to each tube.

8. Please control the level before going to the next step



Run at:

25°C for 10 minutes
42°C for 50 minutes
48°C for 10 minutes
70°C for 10 minutes
4°C for 5 minutes

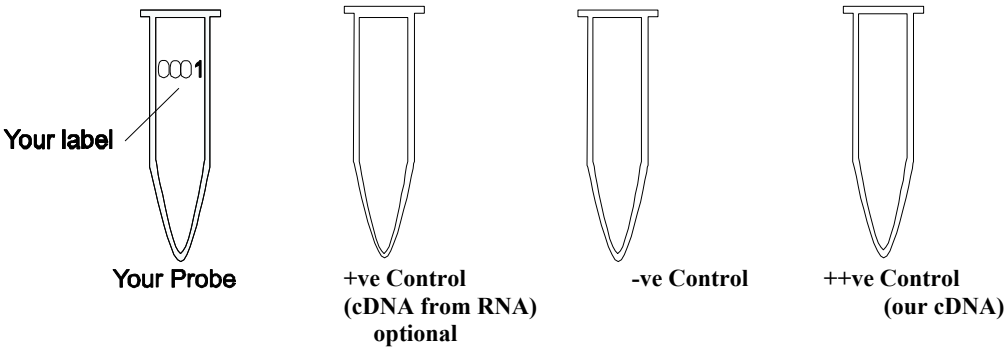
This can be done in Thermocycler.
Now you have got cDNA. Please proceed to PART 2 of the protocol (cDNA can be stored at -20°C).

PART 2 – it is a one step PCR.

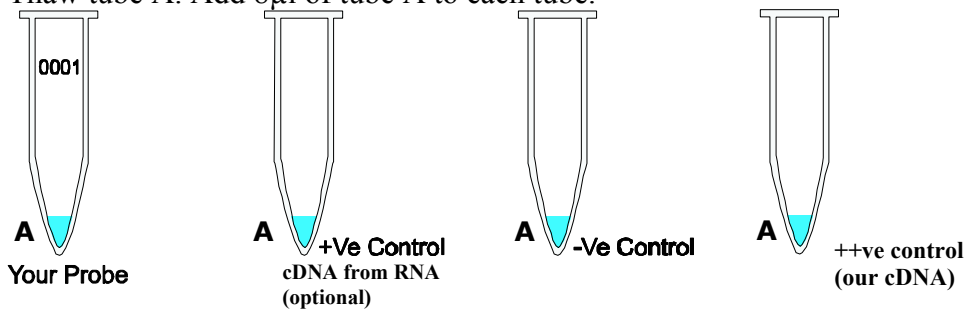
STEP A

1. Kindly thaw **one tube** each of A, B, D1 **(to be made through user. If you do not have this, please run the assay without this)**, D2, E and F. After thawing, kindly put tubes at 4°C (as it is better). If the kit is not in use, store them at -20°C.

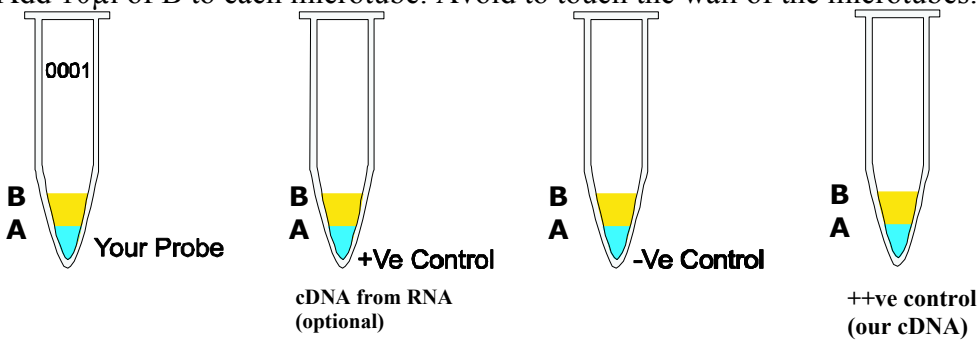
2. Mark your microtubes with a sample number, +ve Control and –ve Control.



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

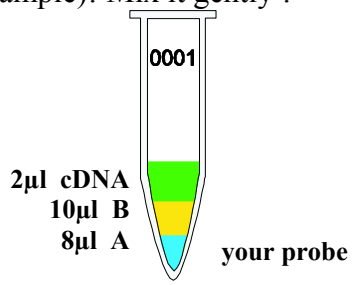


4. Add 10µl of B to each microtube. Avoid to touch the wall of the microtubes.

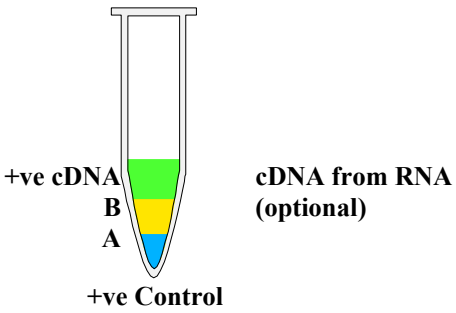


5. **TIP: you can calculate the total requirement of chemicals needed . You need 8µl A + 10µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 80µl of A + 100µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save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

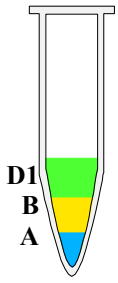
6. Add 2µl of your cDNA template with pipette tip with filter to each microtube according to your label except +Ve and –Ve (Avoid touching the wall). **Use everytime a new pipette tip** (for each sample)! Mix it gently !



7. Use new pipette tip with filter. Add 2µl of +Ve control made through you in the first step (avoid to touch the wall). Use a new pipette tip. Mix it. **This is optinal, hence it is not necessary to perform.**

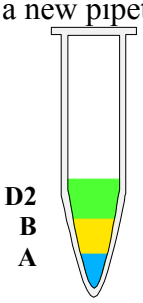


7a. Use new pipette tip with filter. Add 2µl from tube D1.This is positive control supplied with our kit. Mix it. **Important: Ignore this if you do not have this !**



++ve Control (our cDNA)

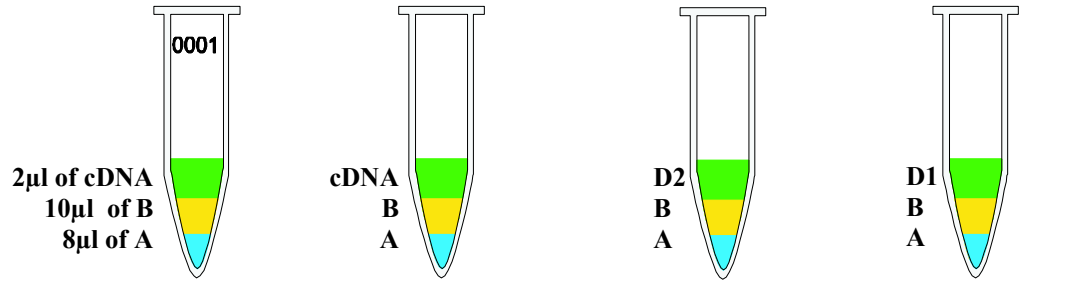
8. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control . Mix it.



-ve Control

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

10. Run the program 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 92°C
2. 45 seconds at 92°C
   45 seconds at 52°C
   45 seconds at 72°C
   } 40 cycles
3. 300 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.

11. After step 10 is finished take out the microtubes and centrifuge for a while.

Now go to STEP B.

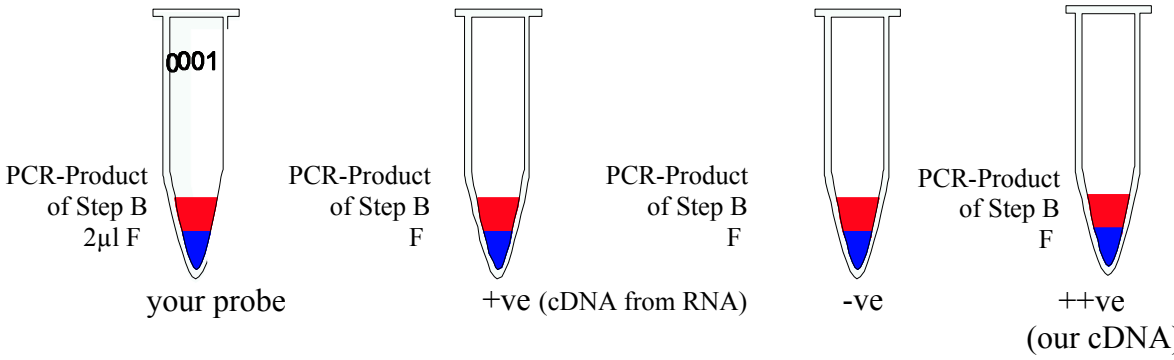
STEP B

1. Prepare the gel Agarose 1.5% in TAE (1x) buffer.

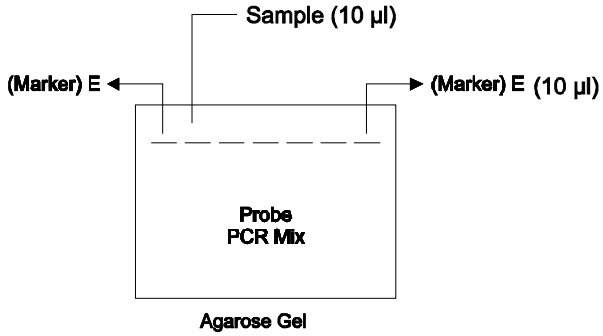
2. Let the Gel dry and add this TAE (1x) buffer in gel chamber.

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 and cDNA Controls).



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 **50 min**. at **120 Volt**.

8. Make staining solution ready.

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

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

11. You must find the bands in positive control and no band in negative control.
You will see band **334bp** in positive control and positive samples for Lassa virus VIRUS.

Recommendation: genesequencing is highly recommended to reconfirm the 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.

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v1.0

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