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Lot-No.

MANUAL
Real time – one step

Ref. FR033

100 Tests (Ready to use PCR kit)

Expiry time: 1 year

STORE AT -20°C

Real time - Measles virus

-Only for in vitro use-
-Only for research use (human)-
-To be used by a technical person-

Principle and use

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

Real time PCR is based on fluorogenic dyes. In our kit we use 2 dyes, they are 6-Carboxy tetramethyl rhodamine (reporter) and Carboxy-fluorescein (quencher). Up to 41 Ct should be taken positive. Value between 42-45 Ct should be taken as marginal positive (doubtful).

This kit needs RNA which can be isolated from blood, serum, cell cultures, vaccine, urine, oral fluid, nervous system, throat probes, lesion probes, tissue 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 (WARNING! THAW THE TUBES SLOWLY: NEVER THAW IN HEATING BLOCK OR WITH HEAT FROM HAND):

It contains the following:

- RNA kit (HX, PF, NTP, RI, RET, DH)
- Tube A (2 tubes)
- Tube B (2 tubes)
- positive (+ve) control (D1): **cDNA – to be stored at -20°C**
- Negative (-Ve) Control (tube D2)

Please check them before you start.

Equipments needed:

- Realtime PCR thermocycler
- Laboratory centrifuge
- microtubes (0.2ml)
- Pipettes with and without filter (20µl, 5µl & 1µl)
- Pipettes (quality pipettes)
- Paper
- Pen
- Microtube
- Ice
- Vortexer
- 96 well PCR plate

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Procedure:

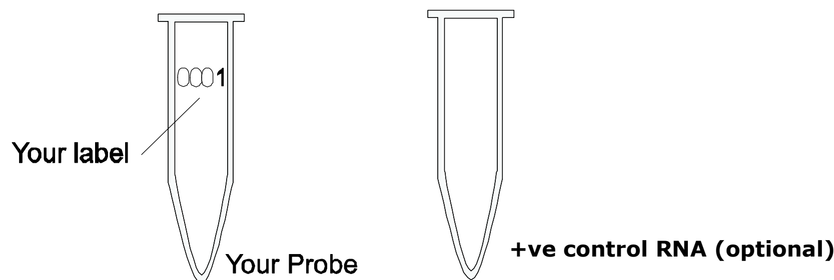
PART 1: Conversion of RNA into cDNA. This should be done with kit, which is with our kit.

ONCE AGAIN:

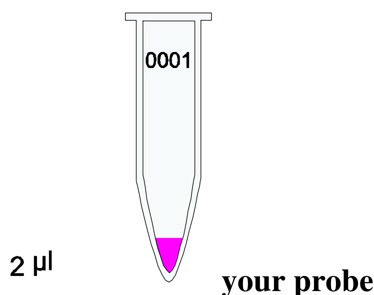
VERY IMPORTANT ! PLEASE USE GLOVES ! DON'T TOUCH ANY OTHER OBJECTS, OTHERWISE THERE MAY BE RNASE CONTERMINATION DURING THIS PART. YOU MUST WORK WITH DNASE- & RNASE-FREE, STERILE PIPETTE-TIPS WITH FILTER. WORK VERY CLEANLY.

STEP A

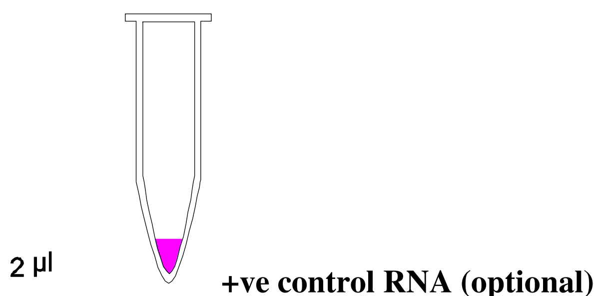
1. Mark your microtubes with a sample number and one with +Ve Control (this +ve control may be made by yourselves, if you have positive material, as we supply only cDNA, **otherwise work without this**).



2. Add 2µl of your isolated RNA from your samples. Sometimes you may have to use 3-4µl (in doubtful cases).



3. Add 2µl of RNA (it can be made by you, if you have any sample of measles virus, which has MEASLES VIRUS as positive control) to +ve control tube. It is optional but not necessary.



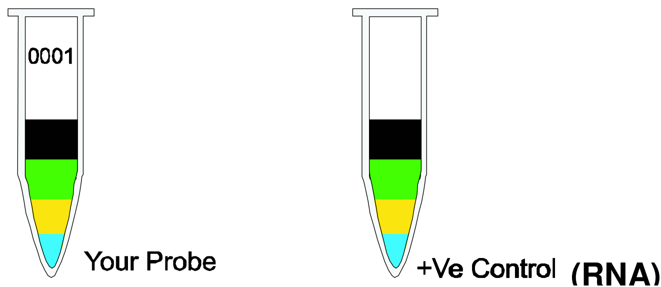
4. Add 1µl of HX (primer) tube and 9µl of DH (water) tube to each tube.

TIP1:	**RNA**	**2µl**
	HX	**1µl**
	DH	**9µl**
		12µl

In order to keep this step small, me must calculate the total requirement, e.g. for 10 probes you need 10µl of HX and 90µl DH.

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).

5. Add: 4µl of PF (buffer)
1µl of RI (enzyme)
2µl of NTP (dNTP-Mix) to each tube
Total: 7µl in tube

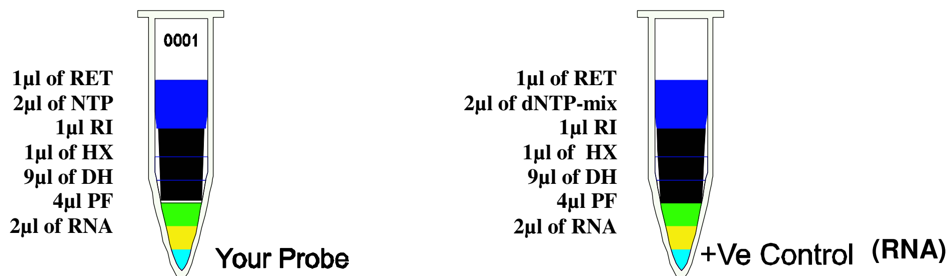


Tip: you can calculate your need for chemicals and mix them together. After that you can add 7µl to each tube (e.g. you want to run 10 reactions, make 70 µl total. Add 7µl to each tube).

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 60 minutes
70°C for 10 minutes
4°C for 5 minutes

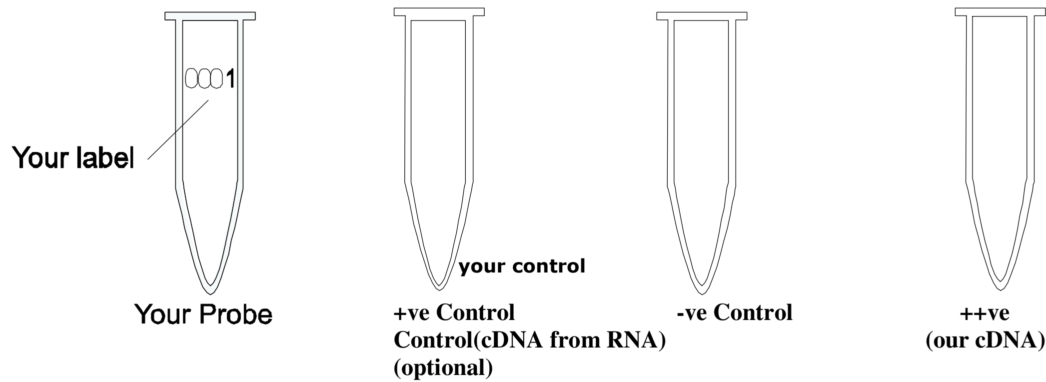
This can be done in the thermocycler. Now you have got cDNA. Please proceed to PART 2 of the protocol (cDNA can be stored at -20°C).

PART 2 – it is a one step PCR.

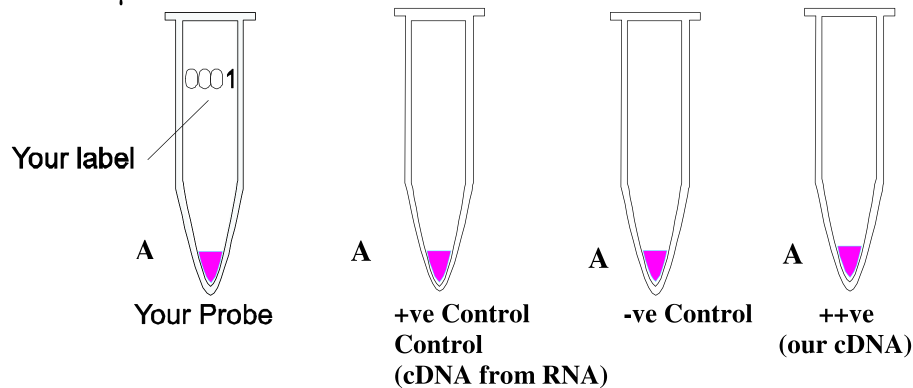
STEP A

1. Kindly thaw one tube A, B, D1 and D2. 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. Kindly keep tubes away from sunlight.

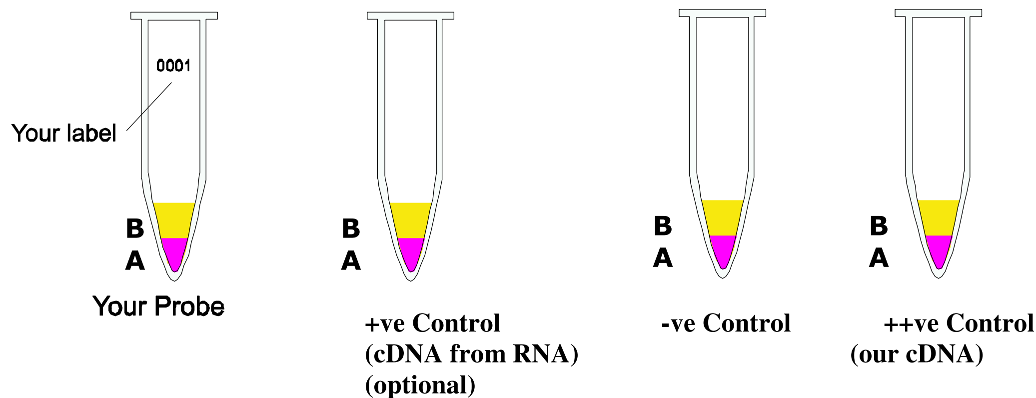
2. Mark your microtubes with a sample number, +ve Control and –ve Control. You can use 96 well microplate instead of tubes.



3. Add 8µl of tube A to each tube.

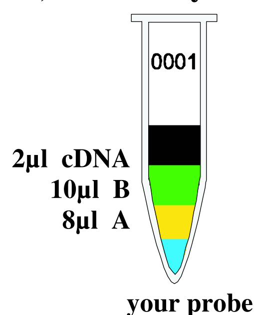


4. Add 10µl of B to each micro tube. Avoid to touch the wall of the microtubes.



TIP: Add 8µl A + 10µl B = 18µl per reaction. In case 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 save time and hardware.

5. Add 2µl of your cDNA template (cDNA made in cDNA-synthesis, see Part 1) with pipette tip with filter to each micro tube according to your label except +Ve and -Ve (Avoid touching the wall). **Use every time a new pipette tip** (for each sample) ! Mix it.



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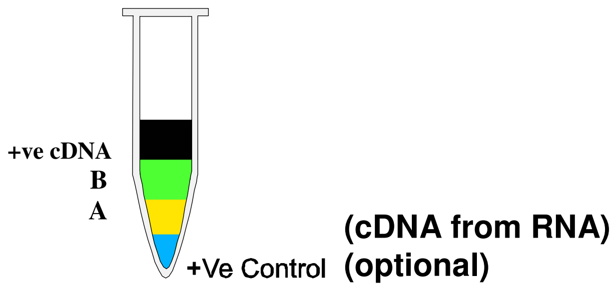
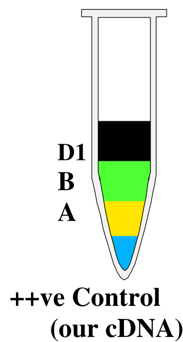
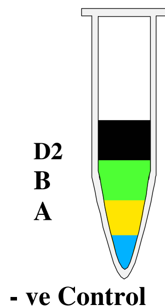
6. Use new pipette tip with filter. Add 2µl of +ve cDNA (made in PART 1) to +Ve Control (avoid to touch the wall). Use a new pipette tip. Mix it.



7. Use new pipette tip with filter.
Add 2µl of cDNA from tube D1. This is the positive control supplied with our kit. Mix it.

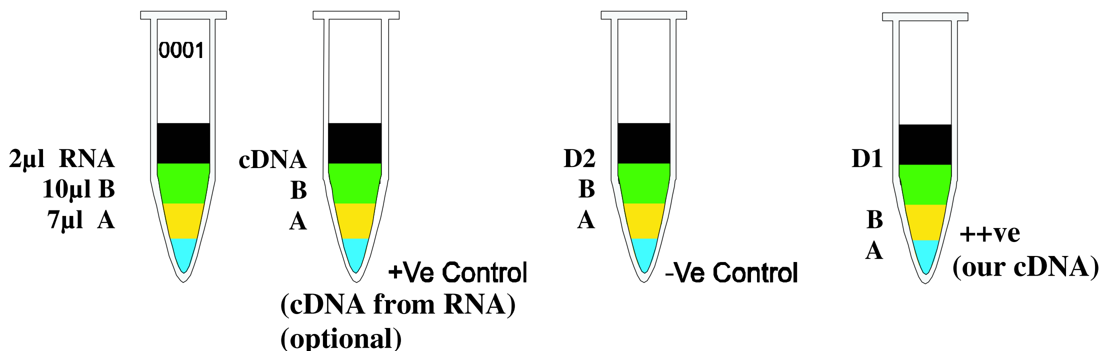


8. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control (don't touch the wall).Mix it.



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 micro tube must be exactly the same.



You must use quencher and reporter dye to setup your software (see FAQ) and run the following program:

15 seconds at 95°C
60 seconds at 60°C } 45 cycles

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

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

STEP B

Once the program will be finished one can see the graphics. The negative control should run along with the bottom and positive control must give a curve in the software graphics. Use your software to analyse 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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FAQ:

1) Q: I cannot find quencher and reporter dye in my software:
   A: Many software has got the words: FAM (as reporter) and TAM (as quencher). Therefore select both in your software.
   If your machines has only one word (for some machines only use the word FAM) you should select this one.