Lot-No.

Ref. SB0031

Expiry date: 1 year

STORE AT -20°C

100 Tests (Ready to use kit)

RT MASTER MIX (cDNA SYNTHESIS KIT)

-Only for in vitro use-
-Only for research use-

Principle and use

This amplification kit has been manufactured by *Genekam Biotechnology AG*, Germany to synthesise cDNA from RNA. cDNA can be used for future analysis.
This kit needs RNA which can be isolated from blood, respiratory swabs, 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.

This kit should be handled with gloves !

Composition:

RNA kit contains the following **(WARNING! THAW THE TUBES SLOWLY: NEVER THAW IN HEATING BLOCK OR WITH HEAT FROM HAND):**

- HX: random hexamer primer
- PF: buffers
- NTP: nucleotides
- RI: Ribonuclease inhibitor
- RET: M-MuLV Reverse transcriptase
- DH: molecular water (2 tubes)
- OP: oligo-dT primer

Please check them before you start.

Equipment needed:

- PCR thermocycler
- Laboratory centrifuge
- UV plateform
- UV safety goggles
- microtubes (0.2ml)
- Pipettes 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:

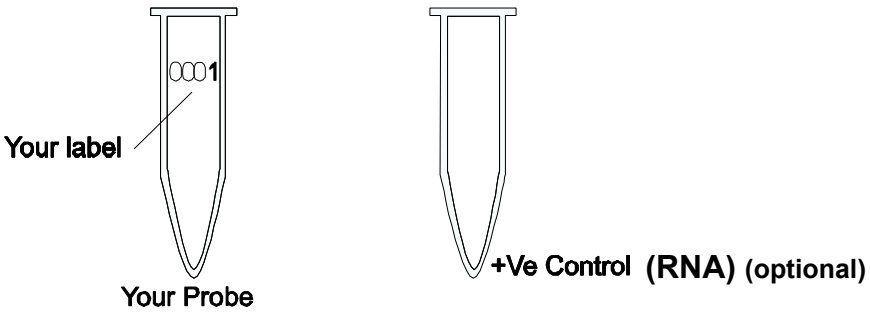
PART 1

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

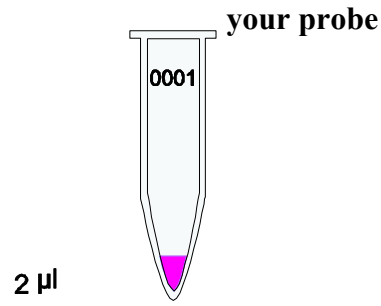
ONCE AGAIN:
VERY IMPORTANT ! PLEASE USE GLOVES ! DON'T TOUCH ANY OTHER OBJECTS, OTHERWISE THERE MAY BE RNASE CONTERMINATION DURING THIS PART.

STEP A

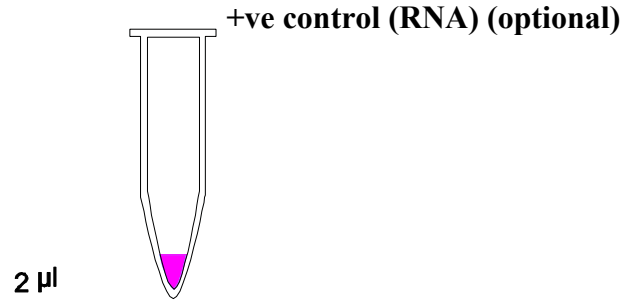
1. Mark your microtubes with a sample number and one with +Ve Control. (RNA), which is optional . It can be made through you.



2. Add 2µl of your isolated RNA from your samples.



3. Add 2µl of RNA as positive control to +ve control tube. (RNA) optional. This is not necessary. It can be made through you as stated in step 1.



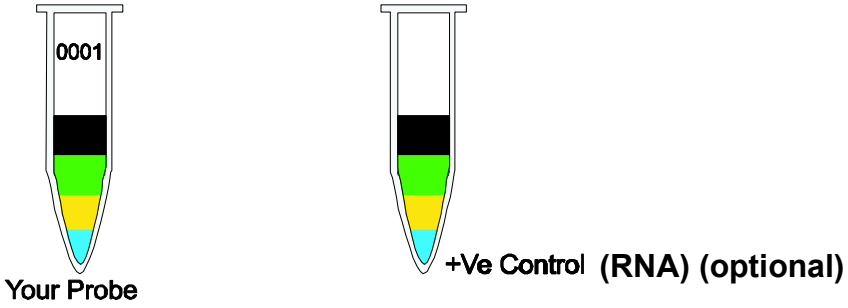
4. Add 1µl of HX and 9µl of DH (water) to each tube.

Precaution: in many publications one needs to add one antisense primer. You can add 1µl of antisense primer instead of HX. In some experiments, eg. tumor marker detection, one can use Oligo-dT-primer (OP). Therefore HX can be replaced through antisense primer or Oligo-dT-primer (OP).

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 (enzyme)
2µl of NTP (nucleotide)
Total: 7µl in tube

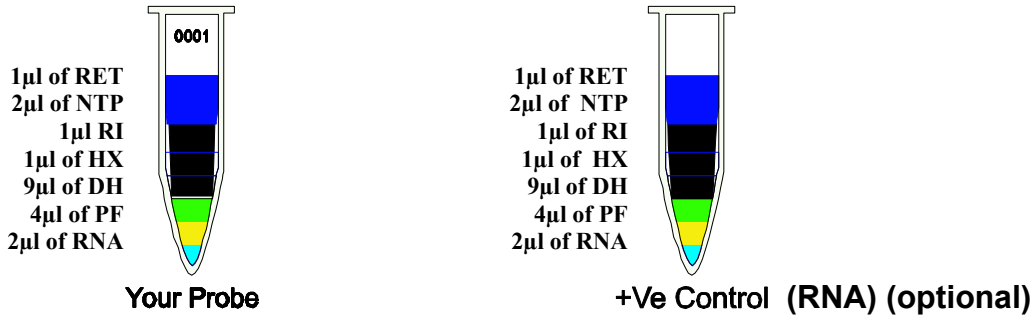


Tip: you can calculate your need for chemicals and mix them together. E.g. for 10 reactions you need 40µl of PF, 10µl of RI and 20µl of NTP = 70µl. After that you can add 7µl to each tube.

6. Run at 25°C for 5 minutes for hexamer **OR** it should be 37°C for 5 minutes for Oligo or antisense primer.

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

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



Run at:

for HX (Hexamer)		**for Oligo or antisense primer**
25°C for 10 minutes		42°C for 50 minutes
42°C for 60 minutes	**OR**	48°C for 10 minutes
70°C for 10 minutes		70°C for 10 minutes
4°C for 5 minutes		cool down to 4°C

This can be done in Thermocycler.
Now you have got cDNA. cDNA can be used for future analysis. cDNA should be store at -20°C.

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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