Lot-No.

Ref. FR271

MANUAL
Real time

Expiry time: 1 year

STORE AT -20°C

100 Tests (Ready to use PCR kit)

Real time - Avian Influenza H9N2

-Only for in vitro use-
-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 *avian influenza virus H9N2* in real time PCR. It is an absolute quantification. This assay carries two different probes in order to cover the vast number of strains of H9N2 circulating around the world and will show H9 and N2 in different channels.

Real time PCR is based on fluorogenic dyes. In this kit, there are 2 probes / dyes, hence you have to program them in your machine:

First Probe: they are 6-Carboxy tetramethyl rhodamine (quencher) and Carboxy-fluorescein (reporter). Up to 36 Ct should be taken positive. Value between 36-40 Ct should be taken as marginal positive (doubtful). This is for N2.

Second probe: they are 6-Carboxy tetramethyl rhodamine (quencher) and HEX (reporter, it is available as VIC in some machines). Up to 36 Ct should be taken positive. Value between 36-40 Ct should be taken as marginal positive (doubtful). This is for H9.

Both channels indicate the presence of H9N2, otherwise they will show H9 or N2, if only one channel is positive.

Hint: If you can use only one dye /channel in your machine (some machine can have only one channel), please program only one channel, which detects only H9. In case of using one channel, it may be covering H9, but not N2.

This kit needs RNA which can be isolated from nasal swabs, blood, urine, respiratory swabs, lung tissue, faecal samples, blood serum, tissue and any body fluid. Kindly use good methods to isolate the RNA.

Safety precautions should be taken as avian influenza virus is infectious for human beings and animals. Always clean your hands before the test use and clean the hands after the test. Wash your face after the test, if possible. Disinfect your working place.

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 **(WARNING! THAW THE TUBES SLOWLY: NEVER THAW IN HEATING BLOCK OR WITH HEAT FROM HAND):**

- Tube A (2 tubes)
- Tube B (2 tubes)
- Tube Y (1 tube)
- Positive (+Ve) Control (tube D1) (1 tube). **This tube must be stored at -20°C.**

• Negative (-Ve) Control (tube D2) (1 tube)

Please check them before you start. **Please store them at -20°C and dark.**

Equipment needed:

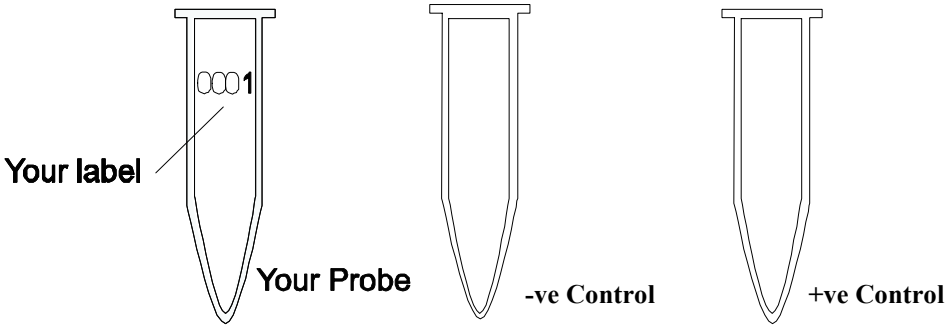
• Laboratory centrifuge
• microtubes (0.2ml)
• Pipette-tips with and without filter (10-100µl & 1-10µl)
• Pipettes (quality pipettes)
• Paper
• Pen
• Vortexer
• 96 well microplates for PCR
• Real time machine

Procedure:

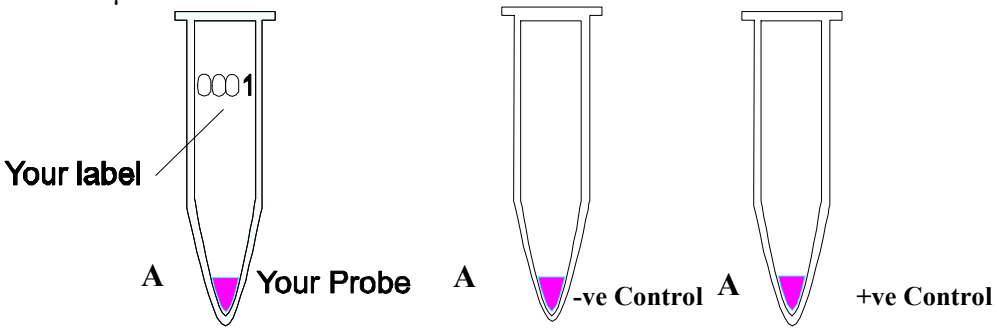
STEP A

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

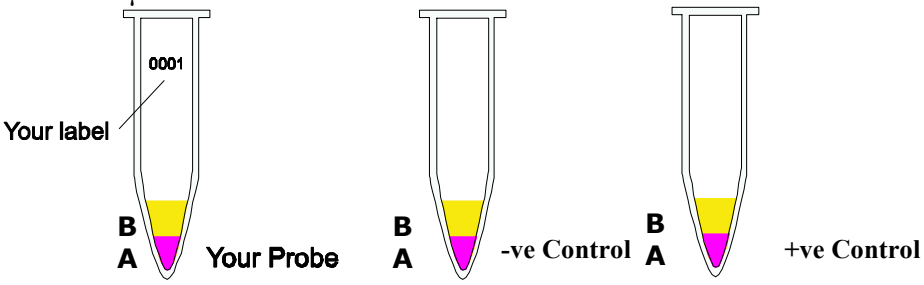
2. Mark your microtubes with a sample number, +ve Control and –ve Control. You can use 96 well microplate instead of tubes.



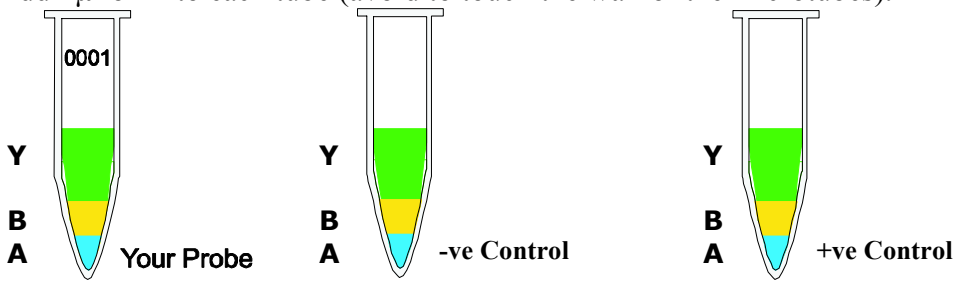
3. Add 7µl of tube A to each tube.



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

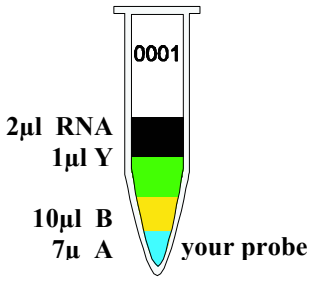


5. Add 1µl of Y to each tube (avoid to touch the wall of the microtubes).

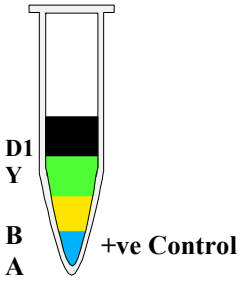


TIP: Add 7µlA + 10µl B + 1µl Y = 18µl per reaction. In case you want to run 10 reactions i.e. you need total 180µl, therefore you should mix 70µl of A + 100µl of B + 10µl of Y = 180µl from which you can take 18µl and add to each tube. This way you save time and hardware.

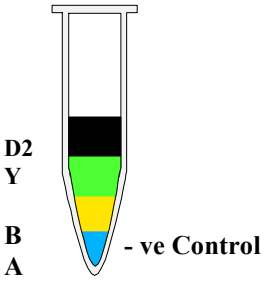
6. Add 2µl of your RNA with sterile pipette-tip with filter to each micro tube according to your label except +Ve and -Ve (Avoid touching the wall). **Use every time a new pipette tip** (for each sample) ! Mix it.



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

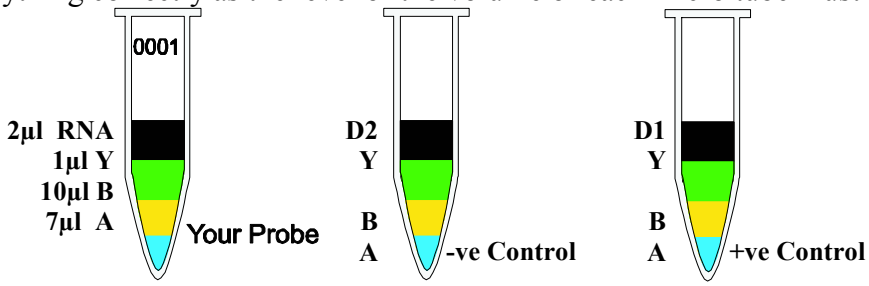


8. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control (don't touch the wall).Mix it.



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 almost the same.



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

1. 60 minutes at 42°C
   10 minutes at 70°C

2. 15 seconds at 95°C
   60 seconds at 60°C
   } x 40 cycles

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.

The results are to be interpreted according to the channels, where they are positive e.g. H9 and N2. If they are positive in both channels, it is H9N2 otherwise it is positive for H9 or N2.

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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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. For HEX, there may be an other channel available in some machines like VIC.