

Lot-No.

MANUAL – Real time

Ref. FR014

Expiry date: 1 year

100 Tests (Ready to use kit)

STORE AT -20°C

MYCOBACTERIUM AVIUM
SP. PARATUBERCULOSIS (Real time)

- Only for in vitro use -
- Only for veterinary use –
- for 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 *Mycobacterium avium sp. paratuberculosis* in real time PCR.

Real time PCR is based on fluorogenic dyes. They are 6-Carboxy tetramethyl rhodamine (reporter) and Carboxy-fluorescein (quencher). Up to 36 Ct should be taken positive or between 36-40 it should be seen as doubtful positive.

This kit needs DNA which can be isolated from milk, blood, respiratory probes, digestive tract tissue, faeces, sputum, lymph nodes (in mammals) spleen, tonsils (in mammals), lungs, tissue and any body fluid. Kindly use good methods to isolate the DNA.

Safety precautions should be taken as *Mycobacterium avium sp. paratuberculosis* 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)
- Positive (+Ve) Control (tube D1) (1 tube)
- 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
- UV platform
- microtubes (0.2ml)
- Pipette-tips with and without filter (20µl, 5µl & 1µl)
- Pipettes (quality pipettes)
- Paper and pen
- Vortexer
- 96 well microplates for PCR
- Real time machine

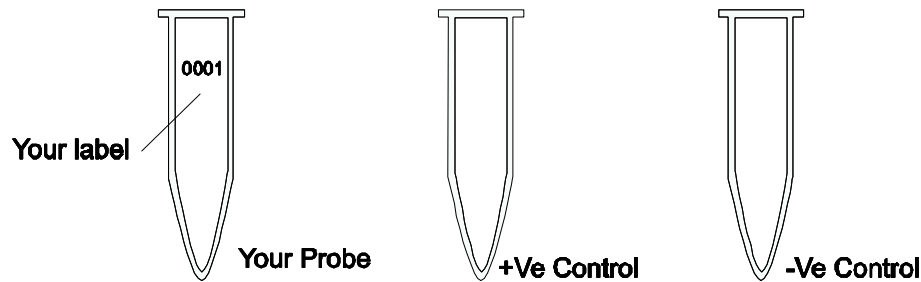
Procedure:

After your DNA isolation is completed. (Kindly use good quality isolation method).
Please go to PCR step

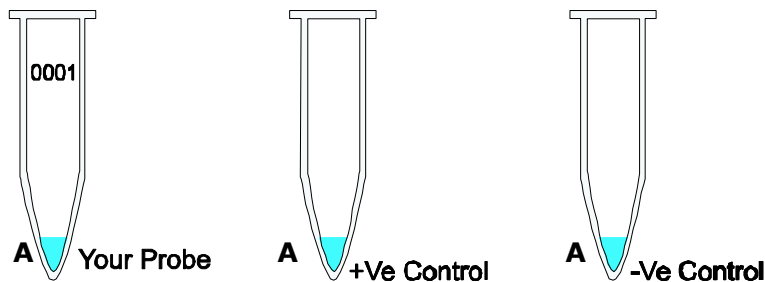
STEP A

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

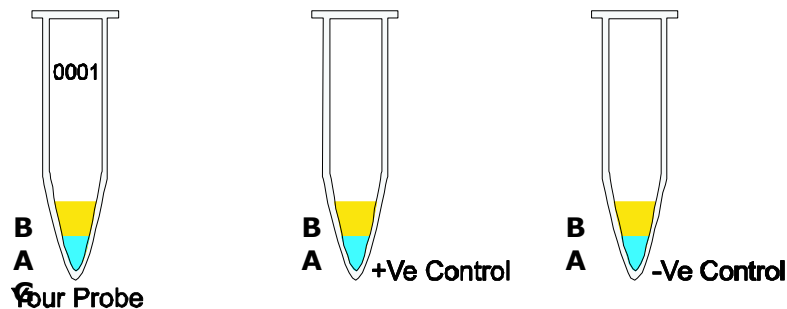
2. Mark your microtubes with a sample number and with +Ve Control and –Ve Control.



3. Thaw tube A. Add 8µl of tube A to each tube. One can also use 96 microwell plate.



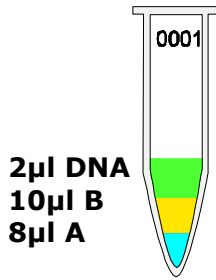
4. Add 10µl of B to each microtube. Avoid to touch the wall of the microtubes.



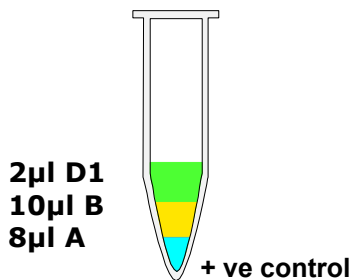
TIP: you can mix 8µl of A + 10µl of B together in one tube (it will be a total volume of 180µl for 10 reactions). From this one can take 18µl and distribute in each tube. In this way one can save the hardware and time.

5. 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).

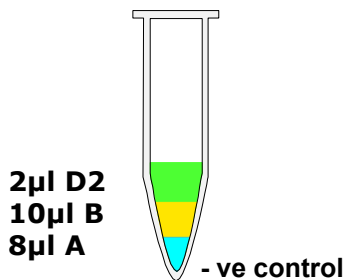
Use everytime a new pipette tip (for each sample)! Mix it.



6. Use new pipette tip with filter. Add 2µl of +Ve (tube D1) to +Ve Control (avoid the wall). Use a new pipette tip. Mix it.

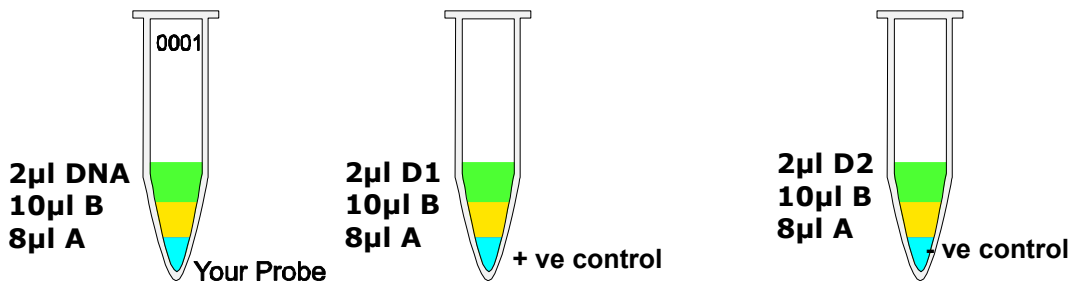


7. Use a new pipette tip. Add 2µl of –Ve (Tube D2) to –Ve Control (avoid the wall). Mix it.



8. Centrifuge all tubes for 20 sec. for 8000 rpm (this is not necessary but it is better). Run PCR now.

9. 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.



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 } 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. Run your PCR now.

10. After step 9 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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v1.2

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>

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.