Lot-No.

MANUAL – one step

Ref. K343

EXPIRY DATE: 1 year

STORE AT -20°C

100 Tests (Ready to use kit)

DIFFERENTIATE BETWEEN MYCOBACTERIUM BOVIS BCG AND STRICT SENSE/NON BCG

-Only for in vitro use-
-Only for veterinary 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 differentiate between Mycobacterium bovis BCG and Mycobacterium (strict sense /non BCG). It needs isolated DNA. Kindly use good methods to isolate.

Kinds of samples: direct detection in tissue, food, water, fodder, blood, faeces and bacterial cultures. This kit can be used to differentiate in samples after first selective enrichment step.

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:

• Tube A (2 tubes)
• Tube B (2 tubes)
• Tube C (2 tubes)
• Positive (+Ve) Control (tube D1) (1 tube)
• 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

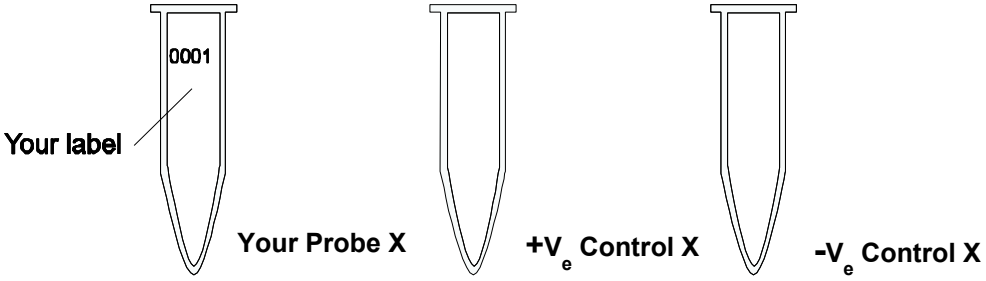
Procedure:

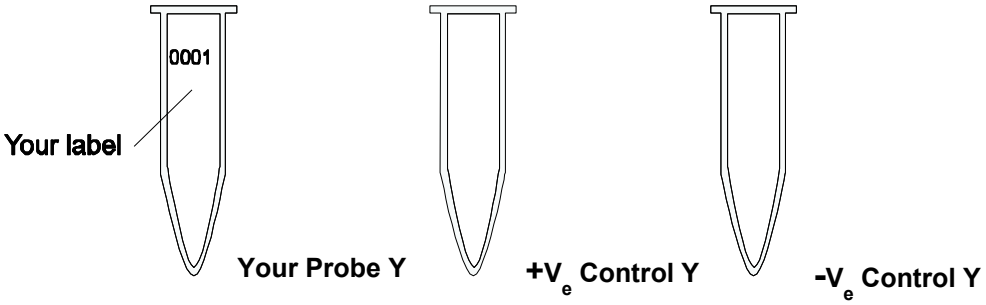
After your DNA isolation is completed. (Kindly use good quality isolation method).

STEP A

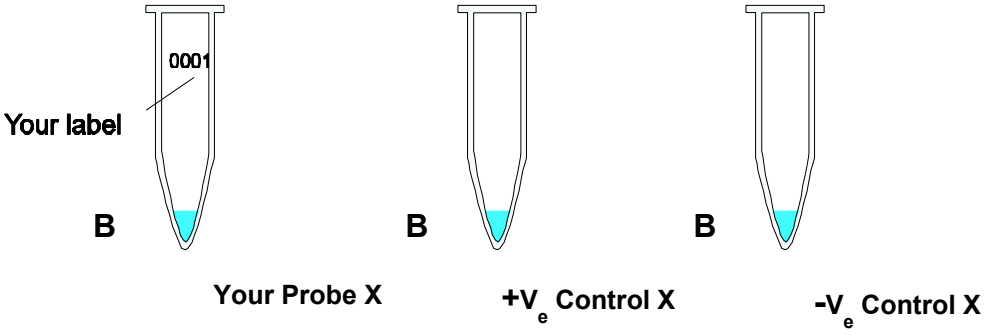
1. Kindly thaw **one tube** each of A, B, C, D1, D2, E and F. After thawing, kindly put the tubes at 4°C (as it is better). If the kit is not in use, store them at -20°C.

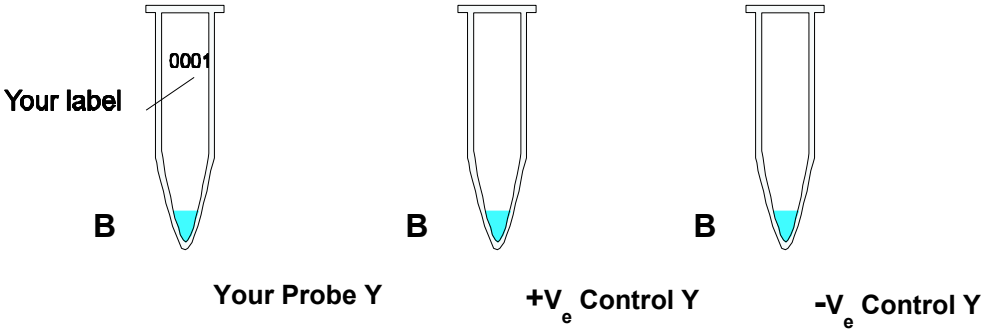
2. Mark your microtubes with a sample number and with +Ve Control and –Ve Control. Divide the samples in two blocks X and Y.



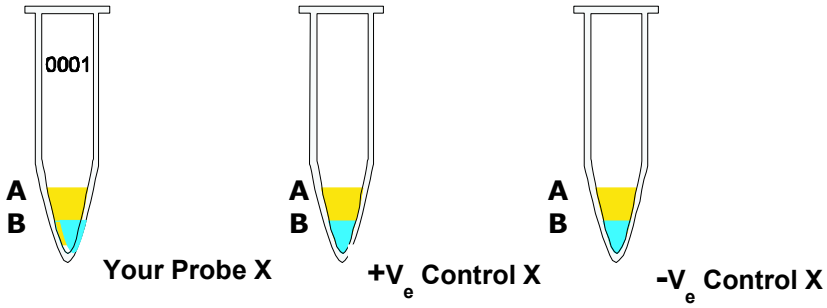


3. Add 10µl of tube B to each tube of both blocks.



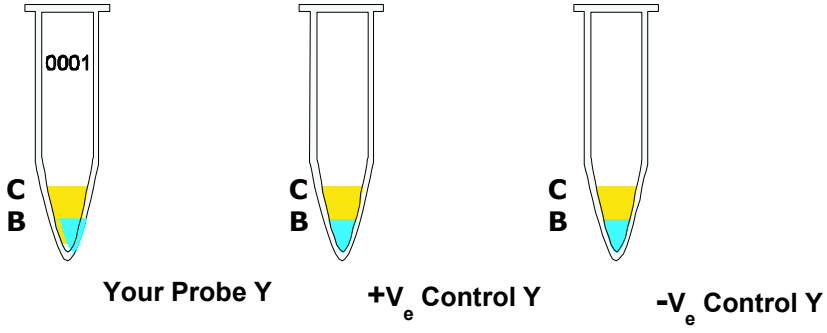


4. Add 8µl of A to each microtube of the block X. Avoid to touch the wall of the microtubes.



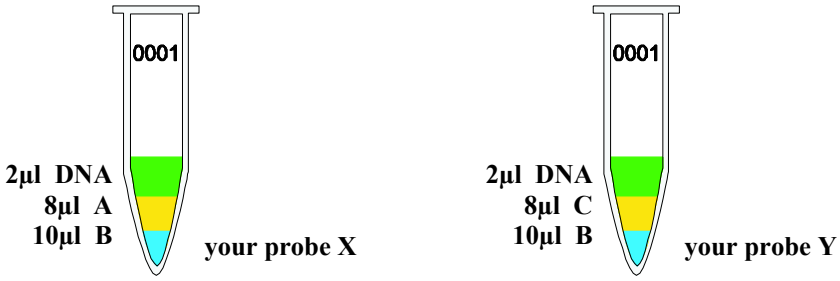
TIP: you can calculate the total requirement of chemicals needed . You need 8µl A + 10µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 80µl of A + 100µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save time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

5. Add 8µl of C to each microtube of the block Y. Avoid to touch the wall of the microtubes.

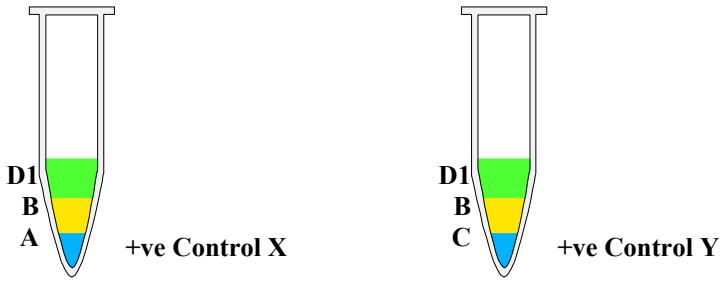


TIP: you can calculate the total requirement of chemicals needed . You need 8µl C + 10µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 80µl of C + 100µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save time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

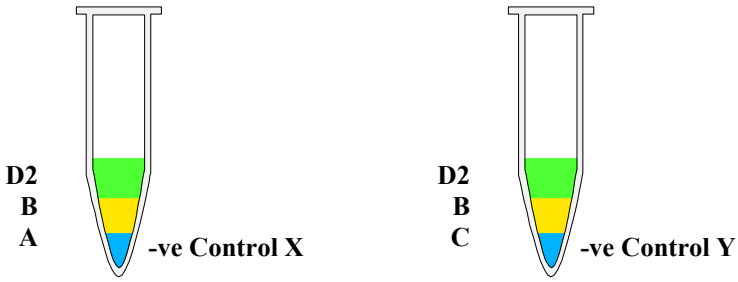
6. Add 2µl of your DNA template (DNA isolated from samples) with pipette tip with filter to each microtube according to your label except +Ve and –Ve controls (Avoid touching the wall).
Use everytime a new pipette tip (for each sample)! Mix it thoroughly.



7. Use new pipette tip with filter. Add 2µl of +Ve (tube D1) to +Ve Control of both block X, and Y (avoid to touch the wall). Use a new pipette tip. Mix it.

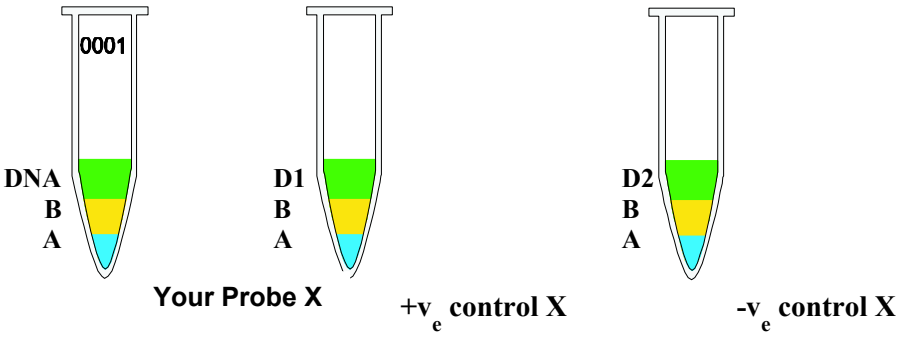


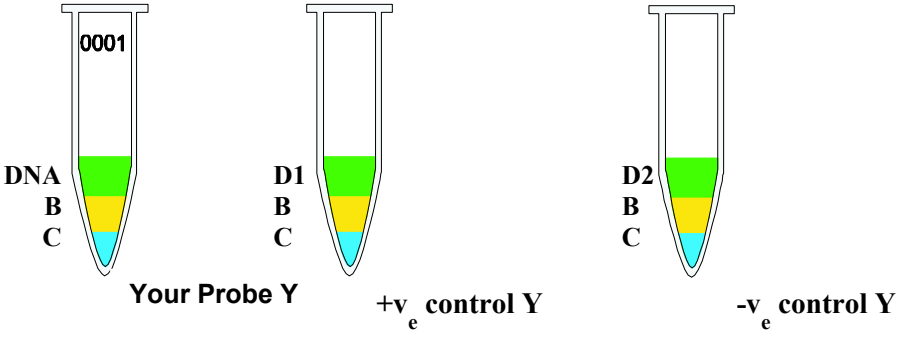
8. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control OF Both blocks x and y . Mix it.



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 microtube must be almost the same.





Now program your PCR machine as follows.

1. 180 seconds at 95°C

2. A. 30 seconds at 94°C
   B. 60 seconds at 65°C
   C. 30 seconds at 72°C
   } 35 cycles

3. 600 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.
Run PCR now.

10. After step 9 is finished take out the microtubes.

To see the results, you can go directly to step gel electrophoresis (STEP B).

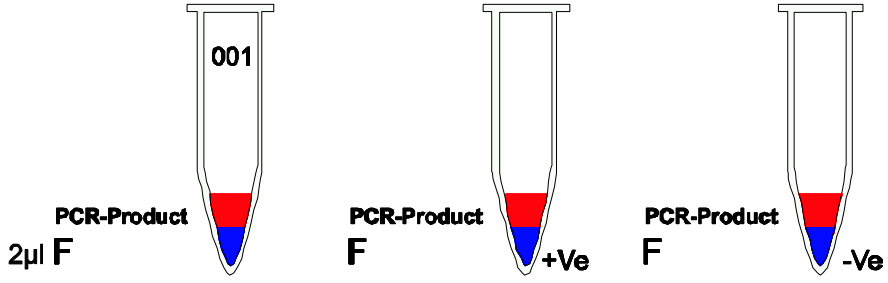
STEP B

1. Prepare the gel Agarose **2%** in TAE (1x) buffer.

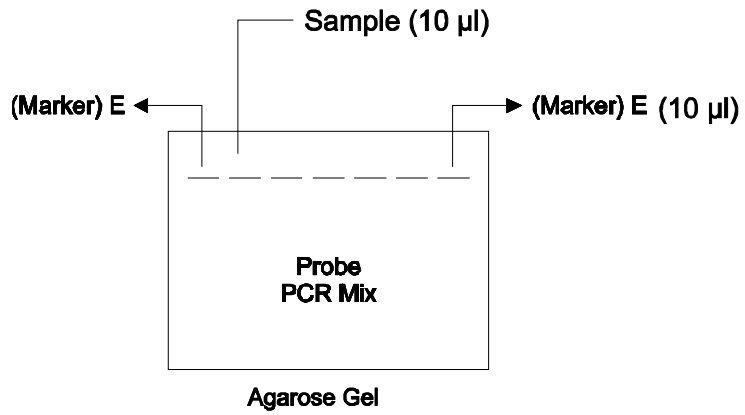
2. Let the gel dry and add this TAE (1x) buffer in gel chamber.

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 Controls) containing PCR product.



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 **45 min**. at **120 Volt**. This may vary.

8. Make staining solution ready.

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

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

11. You must find the bands in +Ve Controls and no bands in –Ve controls. The results will be as follows

Block X

BCG strain 200bp

Block Y

Non BCG 150bp

Recommendation: Gene sequencing is strongly 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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