Lot. No.

MANUAL – one step

Ref. K168

Expiry date: 1 year

100 Tests (Ready to use kit)

STORE AT -20°C

PIGEON CIRCOVIRUS

-Only for in vitro use-

-Only for in veterinary use-

-To be used by a technical person-

Principle and use

This kit needs DNA which can be isolated from feather, blood, serum, tissue and any body fluid. Kindly use good methods to isolate the DNA.

Kindly take microbiological precautions to work cleanly.

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)
- Positive (+Ve) Control (tube D1) (1 tube)
- Negative (-Ve) Control (tube D2) (1 tube)
- Marker (tube E) : 100bp (max. 1000bp) (1 tube)
- Dye (tube F) (1 tube)

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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
- Pen
- Agarose (good quality)
- Staining (Ethium Bromide)
- TAE buffer 1x
- Ice
- Vortexer

Procedure:

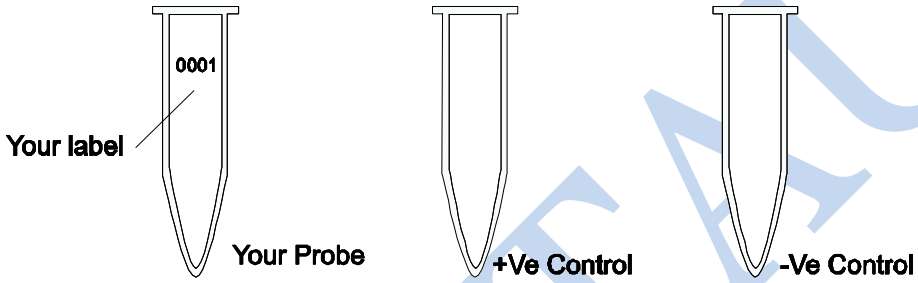
After your DNA isolation is completed. (Kindly use good quality isolation method).

Please go to PCR step

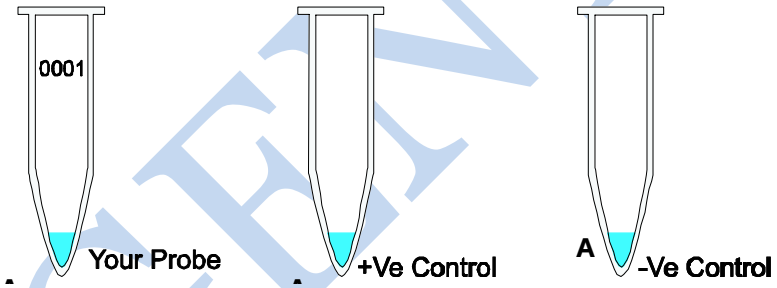
STEP A

1. Kindly thaw one tube each of A, B, D1, D2, E and F. After thawing, kindly put the tubes at 4°C (as it is better). However, you can also work on room temperature. In our laboratory we work in room temperature. However in countries with a high temperature, this precautions should be taken. In case you want to test the probes in coming 2-3 weeks keep them at 4°C, otherwise store them at -20°C.

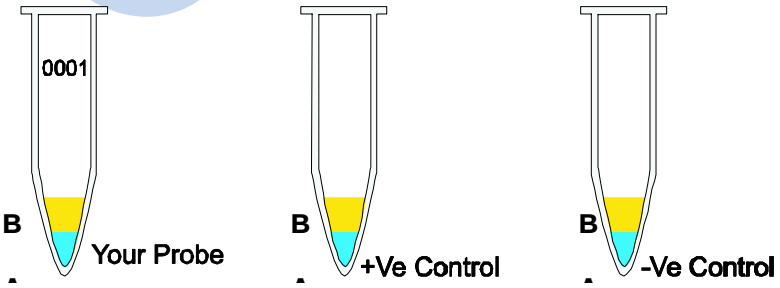
2. Mark your microtubes with a sample number and with +Ve Control and –Ve Control.



3. Thaw tube A. Add 8µl of tube A to each tube.



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



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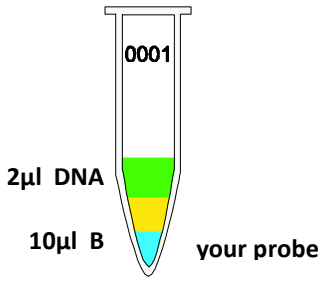
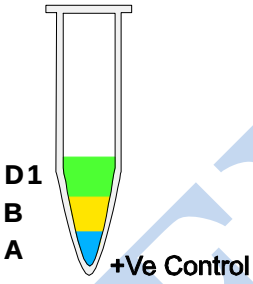
5. **TIP: you can calculate the total requirement of chemicals needed . You need 8µl A + 10µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 80µl of A + 100µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save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

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 (Avoid touching the wall).

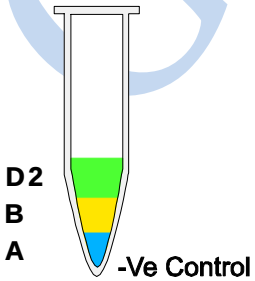
Use everytime a new pipette tip (for each sample)! Mix it.



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



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

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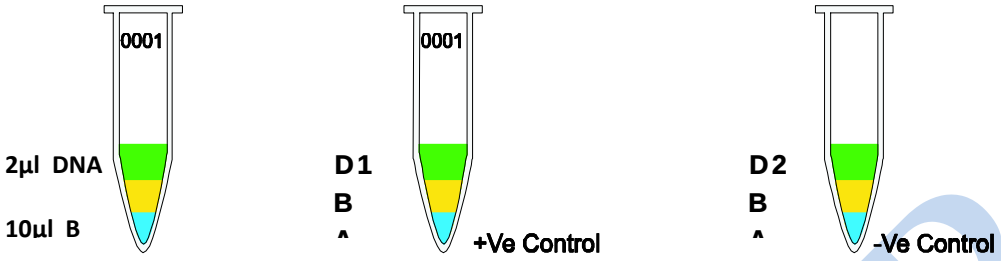
10. Run the program of your thermocycler as followings:

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. 300 seconds at 94°C
2. 30 seconds at 94°C
   30 seconds at 55°C
   30 seconds at 72°C
   } 35 cycles
3. 300 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 your PCR now. Please thaw tube E and F.

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

To see *Pigeon Circovirus*, you can go directly to step gel electrophoresis (STEP B).

STEP B

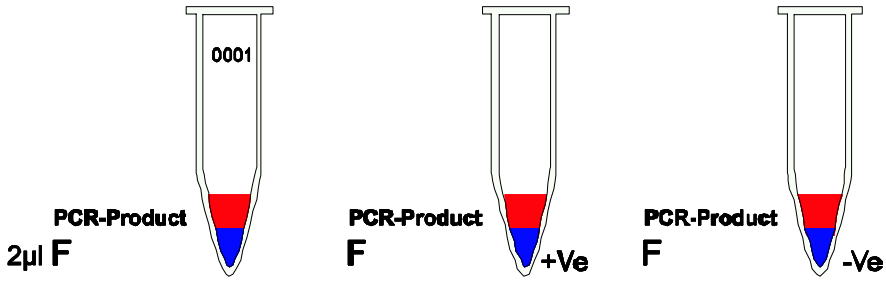
1. Prepare the gel Agarose **1.5%** in TAE (1x) buffer.

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

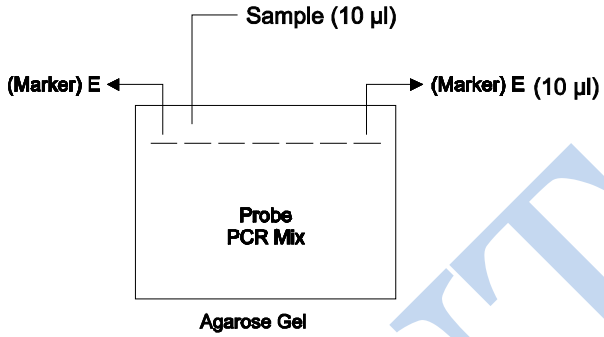
3. Take the tube E (marker). Make ready to use for gel electrophoresis.

4. After the PCR step is finished, now you can prepare for gel Agarose electrophoresis.

Take 2µl of dye (tube F) 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 **50 min**. at **110 Volt**.

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 band in +Ve Controls and no bands in –Ve controls. (**388bp** in +Ve and positive samples for *Pigeon Circovirus*).

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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