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Lot-No.

Ref. K200 **MANUAL** **Expiry date: 1 year**

100 Tests (Ready to use kit) **STORE AT -20°C**

FELINE INFECTIOUS PERITONITIS (FIP) ONESTEP

-Only for in vitro use-
-Only for veterinary use-
-To be used by technical person-

Principle and use

This amplification kit has been manufactured by *Genekam Biotechnology AG*, Germany to detect FIP in nested PCR.

This kit needs RNA which can be isolated from blood, serum, spleen, lymph nodes, 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.

Composition:

It contains the following:

• Tube A (2 tubes)
• Tube B (2 tubes)
• Tube C (2 tubes)
• positive (+ve) control (D1) (1 tube)
• Negative (-Ve) Control (tube D2) (1 tube)
• Marker (tube E) : 100bp (1 tube): 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000bp
• Dye (tube F) (1 tube)
• Tube Y (1 tube)

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, 10µl & 1µl)
• Pipettes (quality pipettes)
• Gel Agarose chamber
• Power supply
• Paper
• Pen
• Agarose (good quality)
• Staining (Ethium Bromide)
• TAE buffer 1x
• Ice

• Vortexer

Procedure:

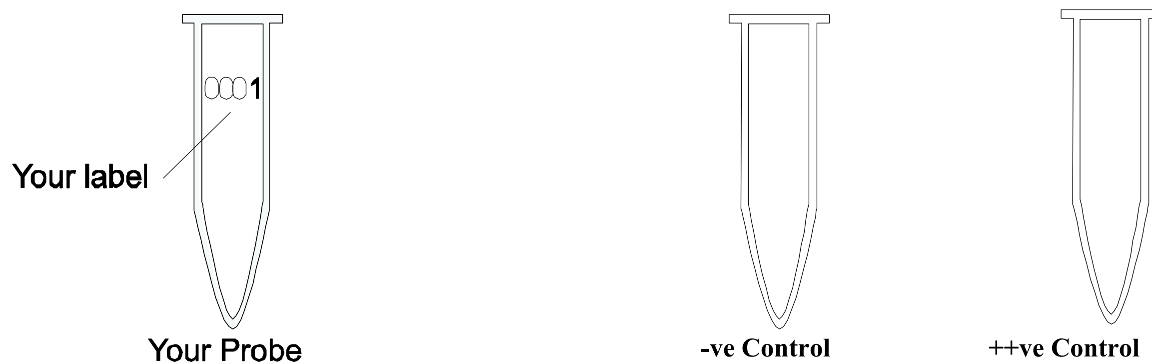
ONCE AGAIN:

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

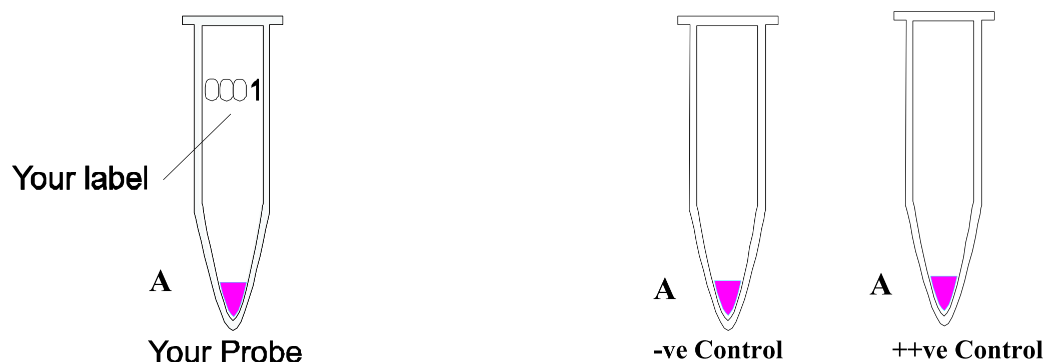
STEP A

1. Kindly thaw **one tube** each of A, B, C, Y, D1, D2, E and F. 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.



3. Add 7µl of tube A to each tube.



4. Add 1µl of tubeY to each tube.

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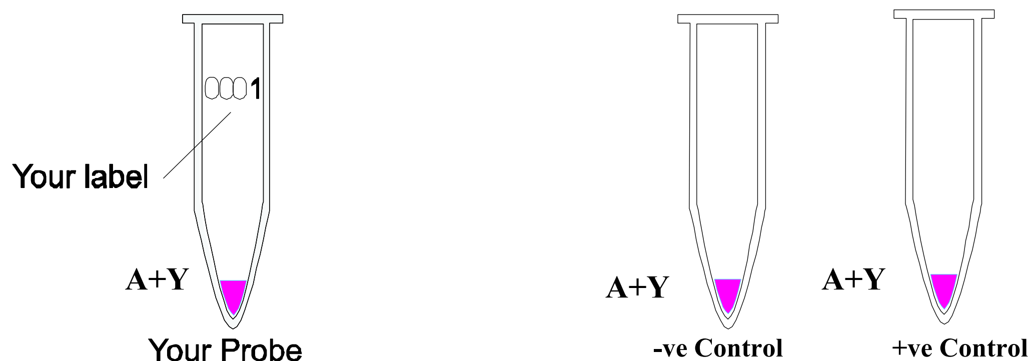
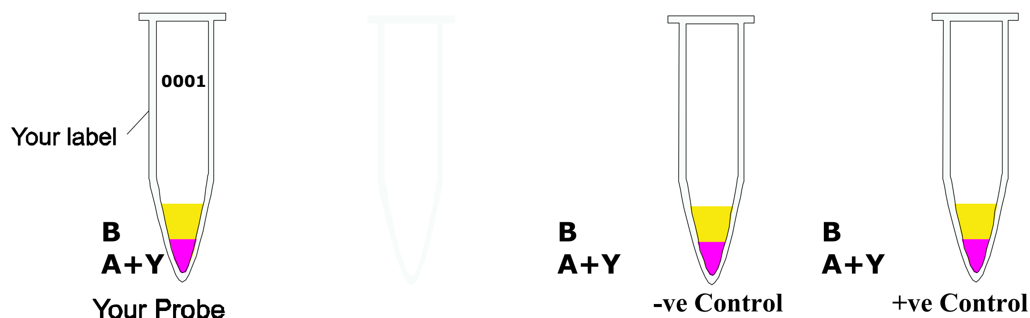
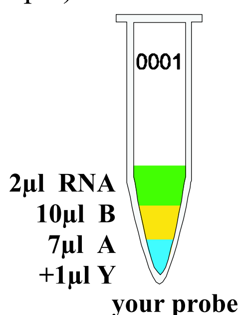


5. Add 10µl of B to each microtube. Avoid to touch the wall of the microtubes.

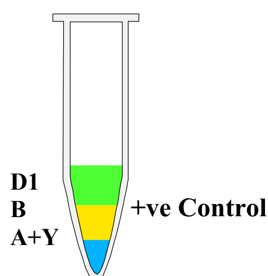


6. **TIP: you can calculate the total requirement of chemicals needed . You need 7µl A + 1 ul Y + 10µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 70µl of A + 10 ul Y + 100µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save time and hardware.**

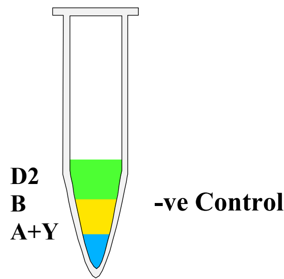
7. Add 2µl of your RNA template with pipette tip with filter to each microtube according to your label except +Ve and -Ve (Avoid touching the wall). **Use every time a new pipette tip** (for each sample)! Mix it.



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

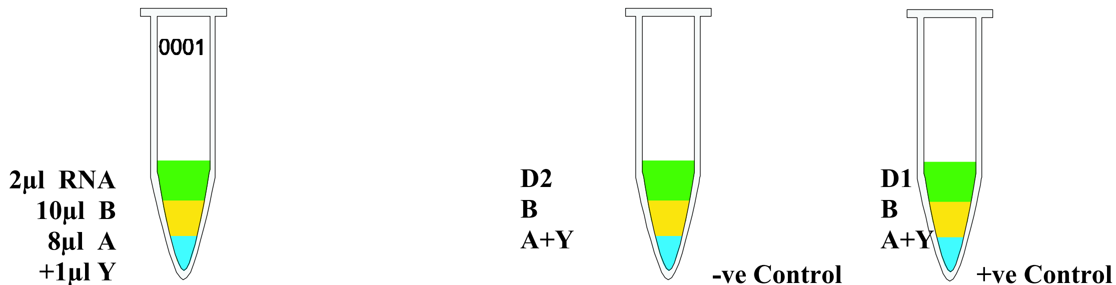


9. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control (don't touch the wall). Mix it.



10. Centrifuge all tubes for 20 sec. for 8000 rpm (this is not necessary but it is better).

11. 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.

A. 120 seconds at 94°C

B. 60 seconds at 94°C
60 seconds at 53°C
180 seconds at 72°C
} 30 cycles

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.

12. After step 10 is finished take out the microtubes and centrifuge for a while.

Now go to STEP C for electrophoresis. In case it is negative or positive in Step A, go to Step B.

STEP B

1. Mark the microtubes with a sample number, +ve Control and –ve Control according to Step A.

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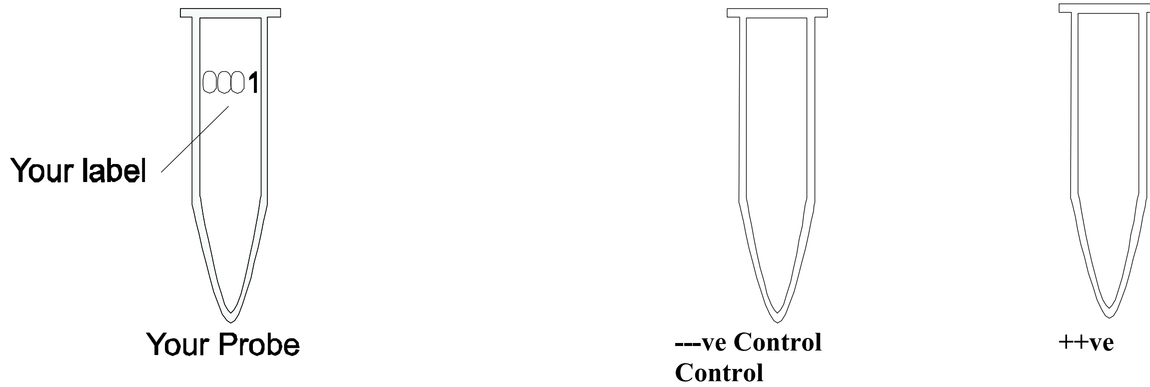
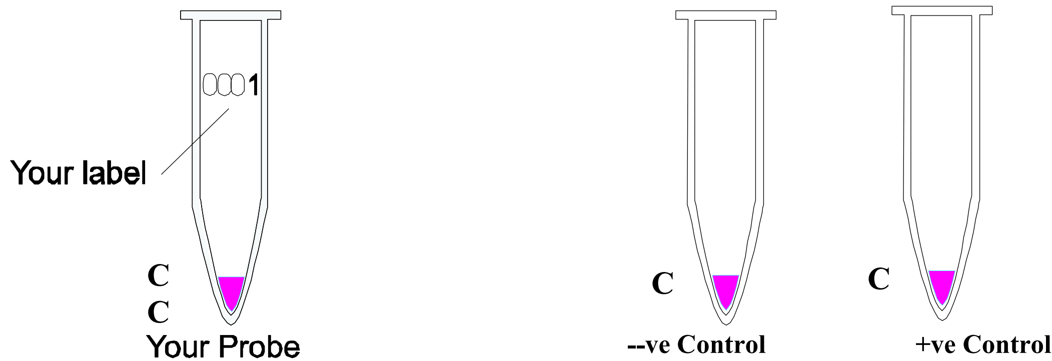
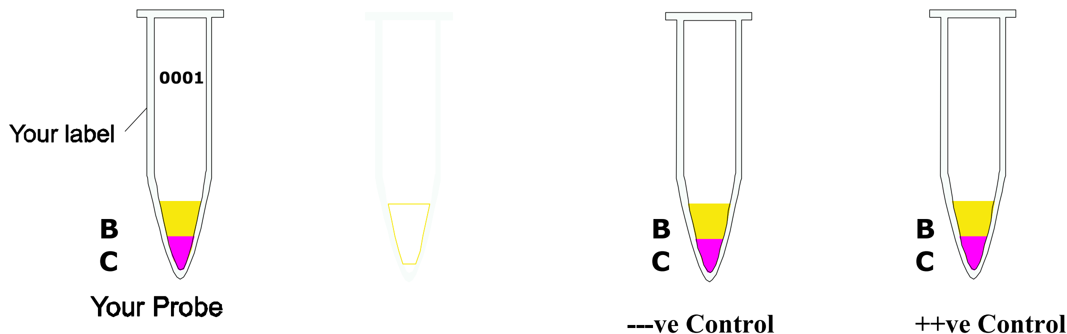


2. Add 14µl of tube C to each tube.



3. Add 5µl of B to each micro tube. Avoid to touch the wall of the microtubes.



4. **TIP: you can calculate the total requirement of chemicals needed . You need 14µl C + 5µl B = 19µl per reaction. You want to run 10 reactions i.e. you need total 190µl, therefore you should mix 140µl of C + 50µl of B = 190µl from which you can take 19µl and add to each tube. This way you can save time and hardware.**

5. Add 1µl of PCR Product of Step A to each tube with corresponding label. Use every time a new pipette tip for each sample. Mix it.

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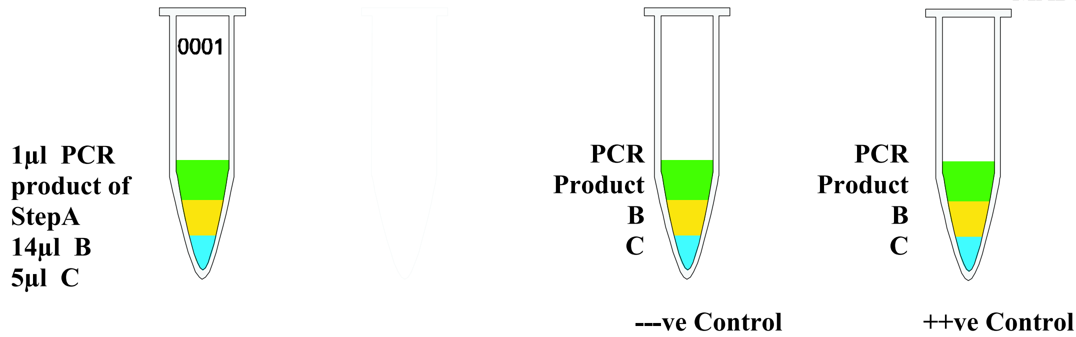


6. Centrifuge all tubes for 20 sec. for 8000 rpm (this is not necessary but it is better).

7. Run the programm 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. 120 seconds at 94°C
2. 60 seconds at 94°C
   60 seconds at 53°C
   180 seconds at 72°C
   } 30 cycles

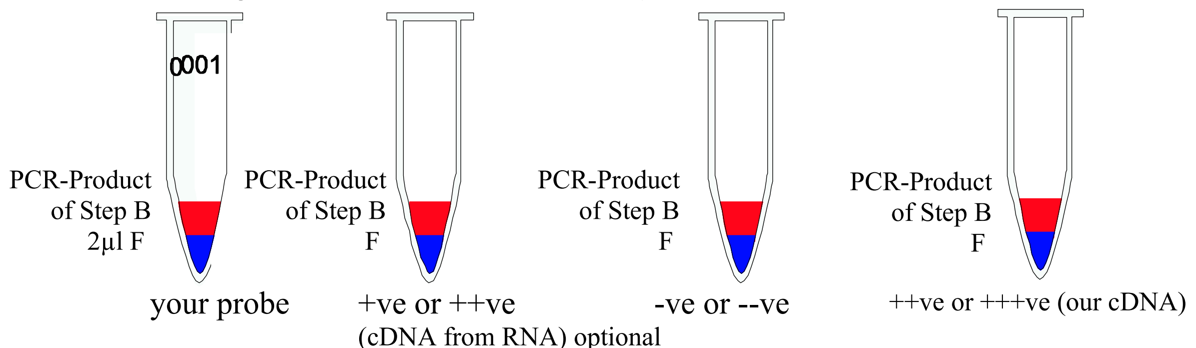
Before you start the PCR program, kindly check whether tubes are closed properly. **Microtubes must be in contact with metal block** (very important!). There should be no air or lose contact with metal block of thermocycler.

8. After step 7 is finished take out the microtubes and centrifuge for a while.

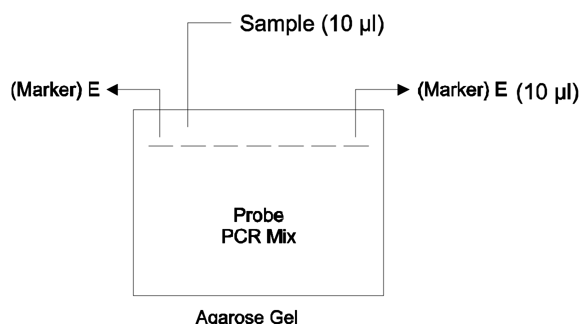
Now go to STEP C for electrophoresis.

STEP C

1. Prepare the gel Agarose 1% 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 and cDNA Controls).



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**.

8. Make staining solution ready.

9. Put the gel for 10-30 minutes staining solution (0.5µg/ml). It is toxic.

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

11. You must find the bands in positive control and no band in negative control.
    1st step: 370bp in positive control as well as in positive samples.
    2nd step:170bp in positive control as well as in positive samples.

HINT:

Sometimes, when you don't find the positive band in the first step, but there is a specific band in the second step, it should be taken as positive.

Recommendation: Gene sequencing is highly recommended to reconfirm the positive 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.** **Last update: 15-12-2011** **v1.1**	**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**