Lot.

MANUAL – one step

Ref. K065

Expiry time: 1 year

STORE AT -20°C

100 Tests (Ready to use kit)

ECHINOCOCCUS GRANULOSUS

-Only for in vitro use-
-Human, only for research use-
-To be used by a technical person only-

Principle and use:

This amplification kit has been manufactured by *Genekam Biotechnology AG*, Germany to detect Echinococcus granulosus in nested PCR (both steps show Echinococcus granulosus), as it checks two times.

This kit needs DNA which can be isolated from blood, tissue, lung tissue, liver tissue, cyst, larvae, stool, part of the parasites and any body fluid. Kindly use good methods to isolate the DNA. Kindly take common safety laboratory precautions during working.

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 (3 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.1000bp): 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000bp
- Dye (tube F) (2 tubes) (1 tube)

Please check them before you start.

Equipment needed:

- PCR thermocycler
- Laboratory centrifuge
- UV plateform
- UV safety goggles
- microtubes (0.2ml)
- Pipettes-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

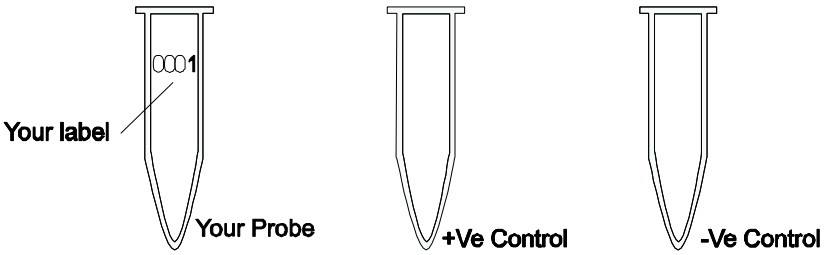
Procedure:

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

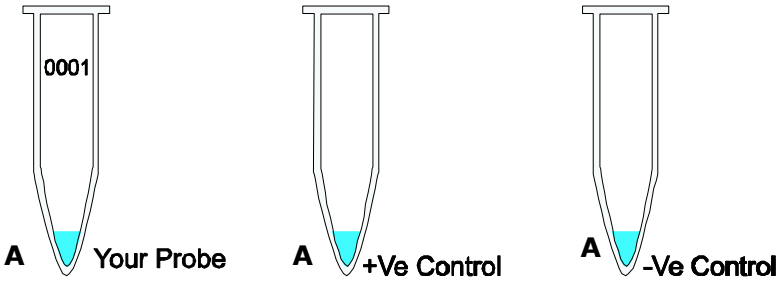
STEP A

1. Kindly thaw **one tube** each: A, 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.

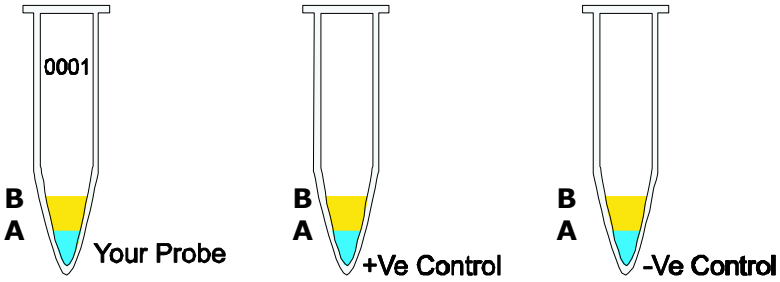
2. Mark your microtubes with a sample number and with +Ve Control and –Ve Control.



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

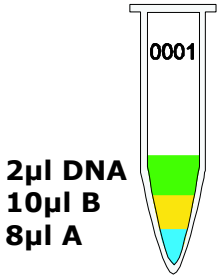


4. Add 10µl of B to each microtube. Avoid to touch the wall of the microtubes.

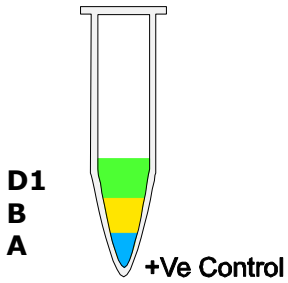


5. **TIP: you can calculate the total requirement of chemicals needed . You need 8µl A + 10µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 80µl of A + 100µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save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

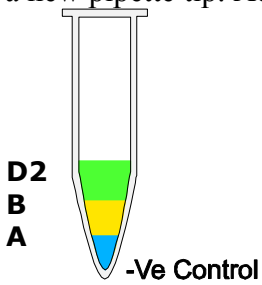
6. Add 2µl of your DNA template (DNA isolated from samples) with pipette tip with filter to each microtube according to your label except +Ve and –Ve (Avoid touching the wall). **Use everytime a new pipette tip** (for each sample)! Mix it.



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.

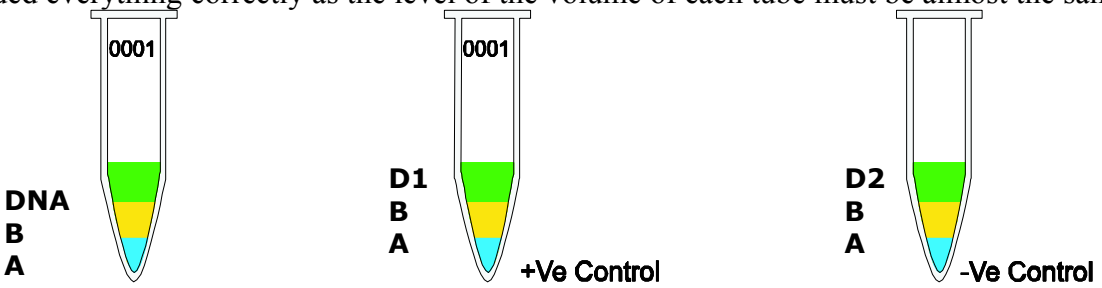


8. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control (don't touch wall).Mix it.



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 tube must be almost the same.



Now program your PCR machine as follows.

1. 360 seconds at 95°C
2. 45 seconds at 94°C
   90 seconds at 55°C
   120 seconds at 72°C
   } 30 cycles
3. 300 seconds at 72°C

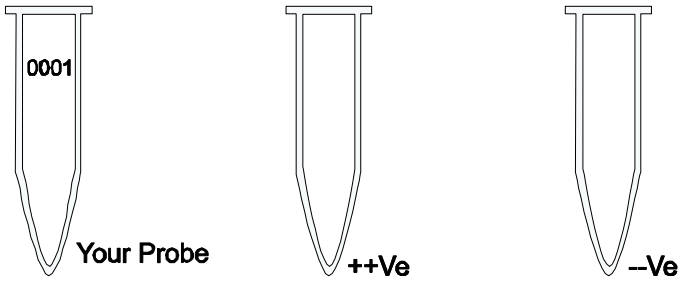
Before you start the PCR program, kindly check whether tubes are closed properly. **Microtubes must be in contact with metal block** (very important!). There should be no air or lose contact with metal block of thermocycler.

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

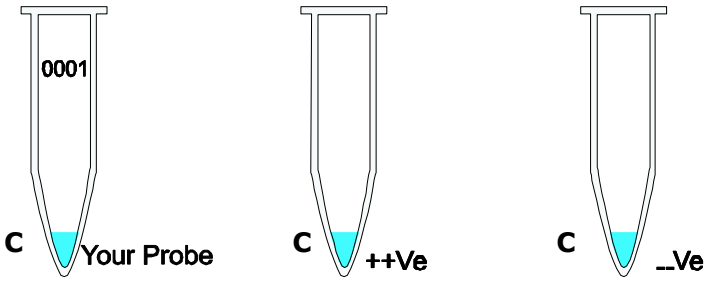
To see Echinococcus granulosus, you can go directly to step gel electrophoreses (STEP C). Meanwhile you can go to step B (This step is for ECHINOCOCCUS GRANULOSUS).

STEP B

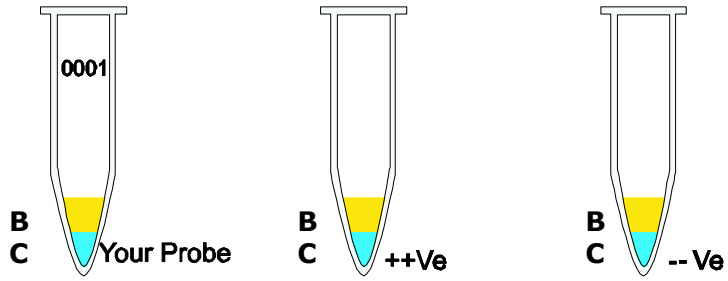
1. Mark your microtubes with a sample number and with +Ve Control and –Ve Control.



2. Add 8µl of tube C to each tube.

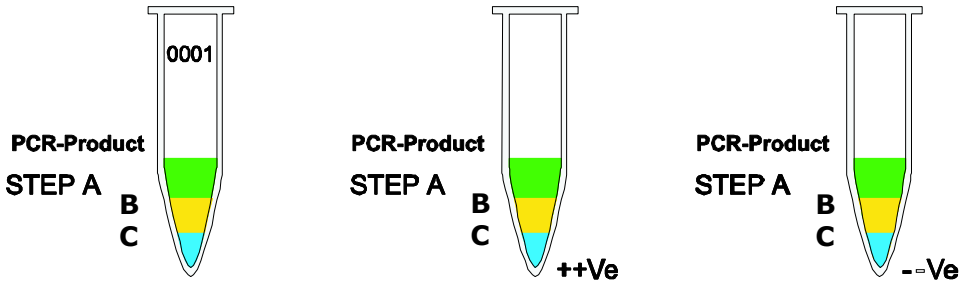


3. Add 10µl of B to each microtube. (Avoid to touch the walls of the microtubes).



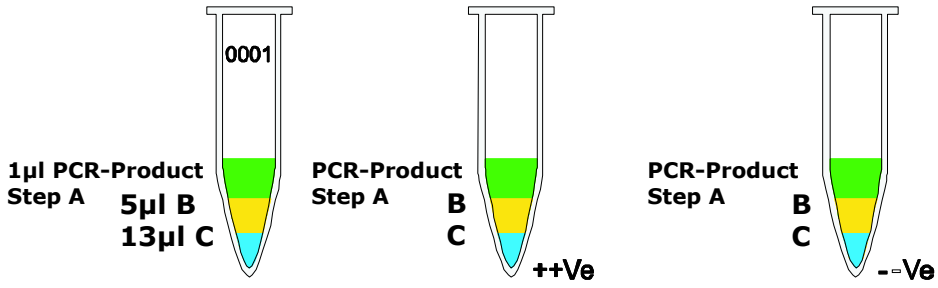
4. **TIP: you can calculate the total requirement of chemicals needed . You need 8µl C + 10µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 80µl of C + 100µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save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

5. Add 1µl of your PCR-product of **STEP A** (sometimes you need to 2µl). It is the end product of thermocycler of **STEP A**) with pipette tip with filter to each (Avoid touching the wall), according to your labelling plan. (Same number or label name as your microtubes of step A). **Use everytime a new pipette tip** (For each sample)! Mix it.



6. Centrifuge each microtube for 20 sec. for 780 rpm (this is not necessary but it is better).

7. Run the program of your thermocycler as followings:
Kindly check whether you have added everything correctly. The level of volume should be the same in each tube.



Program of thermocycler: Run this program

1. 120 seconds at 95°C
2. 30 seconds at 94°C
   45 seconds at 55°C
   60 seconds at 72°C
   } 30 cycles
3. 300 seconds at 72°C

After the program is over you can go to **STEP C**.

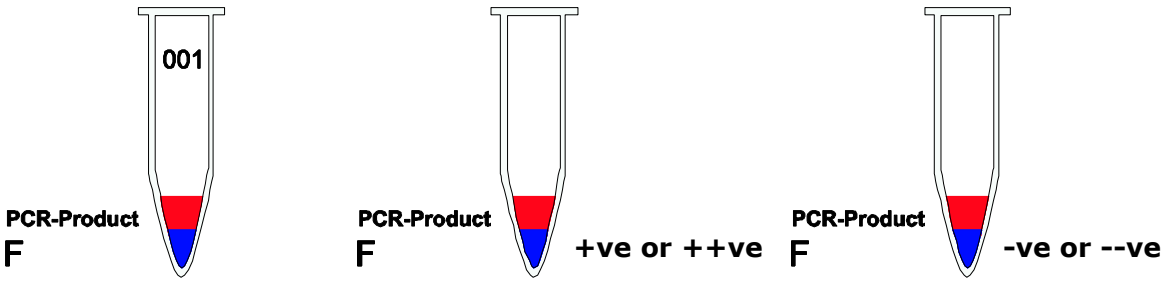
STEP C

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

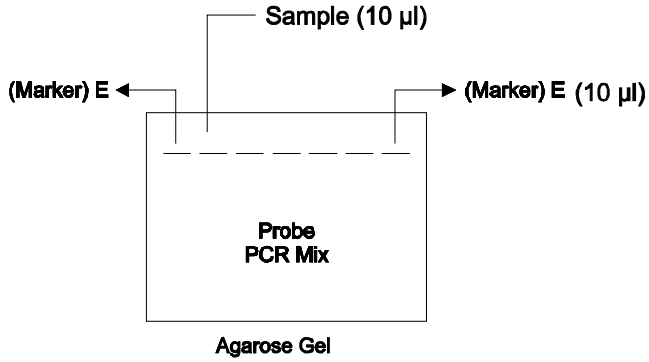
2. Let the Gel dry and add this TAE (1x) buffer in gel chamber.

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) and add to each microtube (with the same number as your PCR microtubes including +Ve & -Ve 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 **40 - 50 min**. at **120 Volt**. It 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 positive control and no band in negative control.
In the first step: **1800bp** in positive samples i.e. it Echinococcus granulosus positive
In the 2nd step: **1000bp** band in positive samples i.e. it is Echinococcus granulosus positive

In 2nd step you can sometimes find more bands. It may be because it is a heavy load of Echinococcus granulosus, as this test checks **two times** for Echinococcus granulosus.

- If you do not see any band in the first step and the 2nd step shows band: it is positive (however you can repeat the test) of Echinococcus granulosus.,
- If you see positive band in the first step and in 2nd step you see more than one band: it is positive (kindly use dilutions of your DNA sample like 1:10 , 1:100 , 1:200 , 1:1000) in order to get single band in 2nd step, so that one can see how strong is the parasite load, such dilutions are not necessary for routine diagnostic use, however you can do this for yourself. In case you need any help please let us know.

Genesequencing 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: 30-01-2012** **v1.4**	**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**