

Lot.

MANUAL – one step

Ref. K015

100 Tests (Ready to use kit)

Expiry date: 1 year

STORE AT -20°C

CHLAMYDIA PNEUMONIAE

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

Principle and use:

This amplification kit has been manufactured by *Genekam Biotechnology AG*, Germany to detect *Chlamydia pneumoniae* DNA (in one step).

This kit needs DNA which can be isolated from blood, respiratory swabs, cardiovascular biopsies, other body tissue and any body fluid. Kindly use good methods to isolate the DNA.

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: (WARNING! THAW THE TUBES SLOWLY: NEVER THAW IN HEATING BLOCK OR WITH HEAT FROM HAND):

It contains the following:

- Tube A (2 tubes)
- Tube B (1 tube)
- 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 platform
- 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

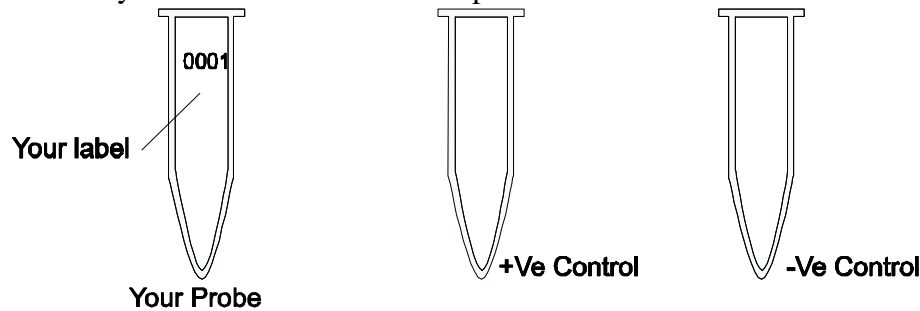
Procedure:

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

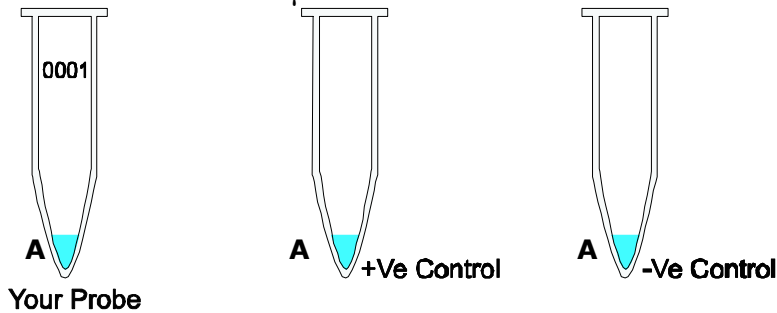
STEP A

1. Kindly thaw one tube each of A, B, D1, D2, E and F. After thawing, kindly put tubes at 4°C (as it is better). However, you can also work at room temperature (as we do in our laboratory). If the kit is not in use, store them at -20°C.

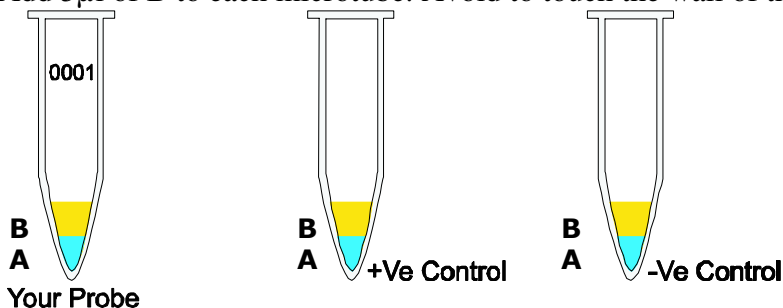
2. Mark your microtubes with a sample number and with +Ve Control and -Ve Control.



3. Thaw tube A. Add 13µl of tube A to each tube.



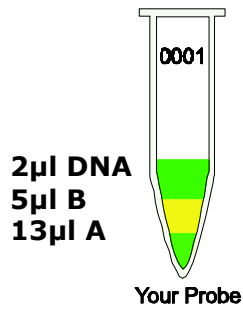
4. Add 5µl of B to each microtube. Avoid to touch the wall of the microtubes.



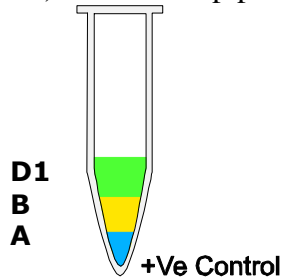
TIP: you can calculate the total requirement of chemicals needed . You need 13µl A + 5µl B = 18µl per reaction. You want to run 10 reactions i.e. you need total 180µl, therefore you should mix 130µl of A + 50µl of B = 180µl from which you can take 18µl and add to each tube. This way you can save time and hardware.

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).

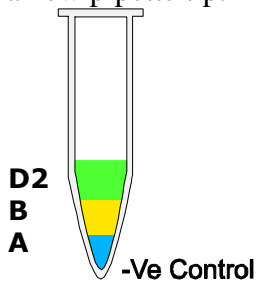
Use everytime a new pipette tip (for each sample)! Mix it.



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

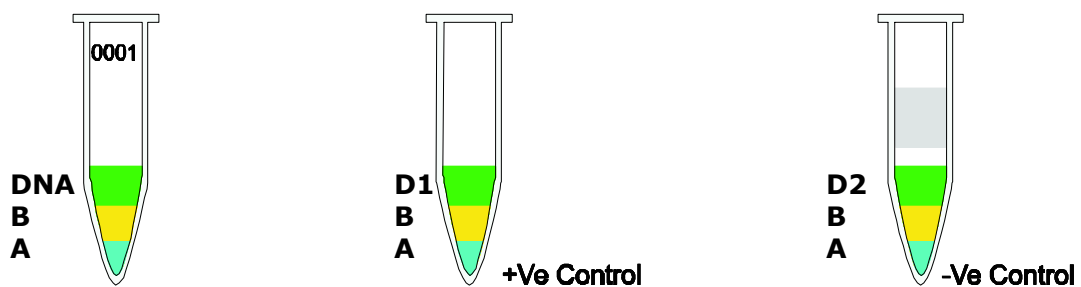


7. Use a new pipette tip. Add 2µl of -Ve (Tube D2) to -Ve Control (avoid the wall). Mix it.



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

9. Run the program of your thermocycler as followings: Kindly check whether you have added everything correctly as level of the volume of each microtube must be almost the same.



Now program your PCR machine as follows.

Step-No.

01.	75 seconds at 95°C	
02.	A. 45 seconds at 94°C	} 4 cycles
	B. 45 seconds at 62°C	
	C. 60 seconds at 72°C	
03.	A. 45 seconds at 94°C	} 4 cycles
	B. 45 seconds at 61°C	
	C. 60 seconds at 72°C	
04.	A. 45 seconds at 94°C	} 4 cycles
	B. 45 seconds at 60°C	
	C. 60 seconds at 72°C	
05.	A. 45 seconds at 94°C	} 4 cycles
	B. 45 seconds at 59°C	
	C. 60 seconds at 72°C	
06.	A. 45 seconds at 94°C	} 4 cycles
	B. 45 seconds at 58°C	
	C. 60 seconds at 72°C	
07.	A. 45 seconds at 94°C	} 4 cycles
	B. 45 seconds at 57°C	
	C. 60 seconds at 72°C	
08.	A. 45 seconds at 94°C	} 4 cycles
	B. 45 seconds at 56°C	
	C. 60 seconds at 72°C	
09.	A. 45 seconds at 94°C	} 4 cycles
	B. 45 seconds at 55°C	
	C. 60 seconds at 72°C	
10.	A. 45 seconds at 94°C	} 4 cycles
	B. 45 seconds at 54°C	
	C. 60 seconds at 72°C	
11.	A. 45 seconds at 94°C	} 4 cycles
	B. 45 seconds at 53°C	
	C. 60 seconds at 72°C	
12.	A. 45 seconds at 94°C	} 20 cycles
	B. 45 seconds at 52°C	
	C. 60 seconds at 72°C	

In above said thermocycler program you must go down in point B from 62 to 52°C.i.e. going down 1 °C in each step and each step must be 4 cycles that is 40 cycles till you reach the temperature of 52°C in the last step. In the last step you must perform 20 cycles i.e. 60 cycles in total thermocycler program i.e. three hours and twelve minutes.

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.

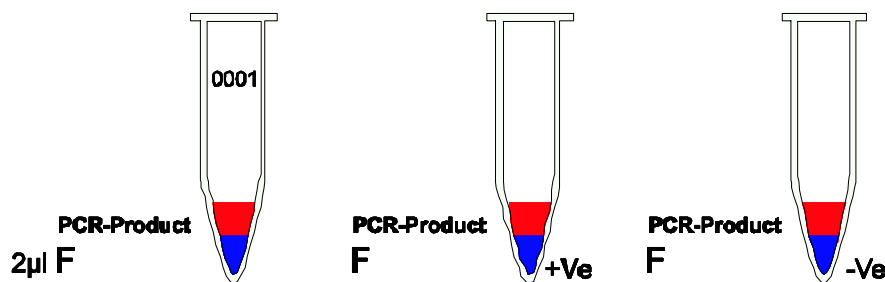
Run PCR now.

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

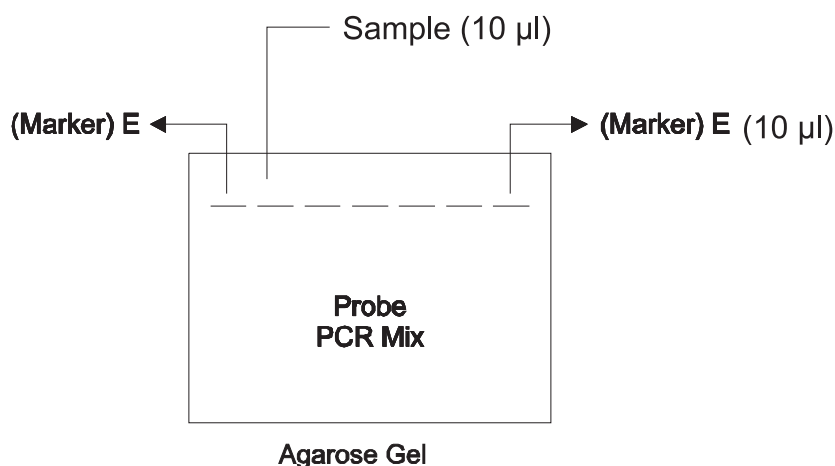
To see Chlamydia pneumoniae DNA, you can go directly to step gel electrophoresis (STEP B).

STEP B

1. Prepare the gel Agarose 2.0% 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 **60 min.** at **120 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 bands in +Ve Controls, positive samples and no bands in –Ve controls. (197 bp in +Ve Control Chlamydia pneumoniae DNA).

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.

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