Lot.

MANUAL – one step

Ref. K410B

Expiry time: 1 year

STORE AT -20°C

100 Tests (Ready to use kit)

WEST NILE VIRUS

-Only for in vitro use-
-Only for research use (human)-
- Only for veterinary use-
-To be used by a technical person -

Principle and use:

This amplification kit has been manufactured by *Genekam Biotechnology AG*, Germany to detect mosquito borne flaviviruses in step A and *West nile virus step B*.

This kit needs RNA which can be isolated from blood, serum, plasma, mosquito, tissue, and any body fluid. Kindly use good methods to isolate the RNA. **Kindly take common safety laboratory precautions during working as westnile virus is infectious and there is no therapy for it.**

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): **it should be stored at -20°C.**
• Positive (+Ve) Control (tube D3) (1 tube): **it is not included, to be made from user from positive material.**
• Negative (-Ve) Control (tube D2) (1 tube)
• Marker (tube E) (1 tube) (max. 1000bp): 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 bp
• Dye (tube F) (1 tube) (1 tube)
• Tube Y (1 tube) (2 tubes)

Please check them before you start.

Equipments needed:

• PCR thermocycler
• Laboratory centrifuge
• UV plateform
• UV safety goggles
• microtubes (0.2ml)
• sterile 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:

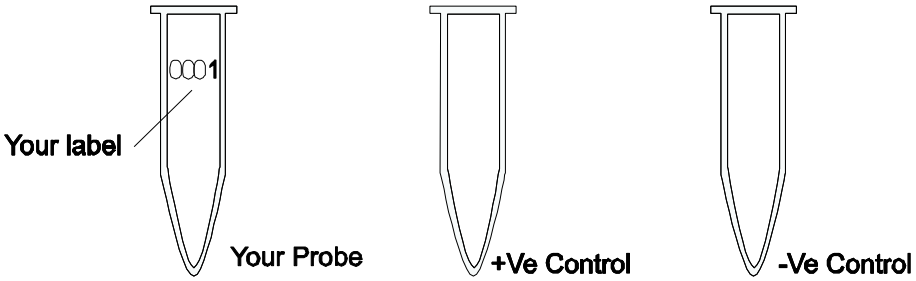
After your RNA isolation is completed. (Kindly use good quality isolation method).

VERY IMPORTANT: WORK VERY CLEANLY AND WITH RNASEFREE, STERILE PIPETTETIPS. DO NOT TOUCH OTHER OBJECTS LIKE TABLES, PENS, CHAIRS DURING THE WORK.

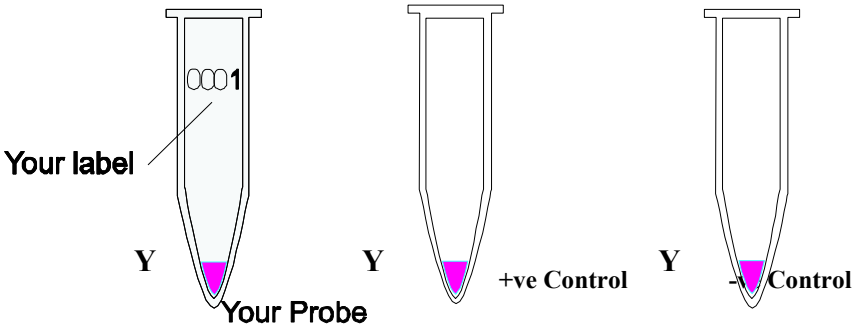
STEP A: In this step, you will detect different kind of mosquito borne flaviviruses like dengue viruses, west nile viruses, Japanese encephalitis virus.

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

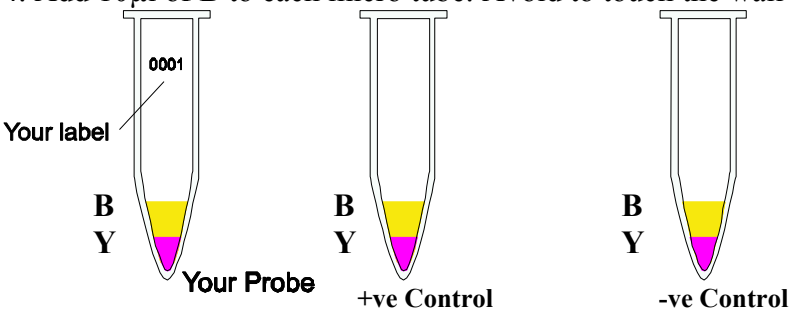
2. Mark your microtubes with a sample number and with +Ve Control and –Ve Control.



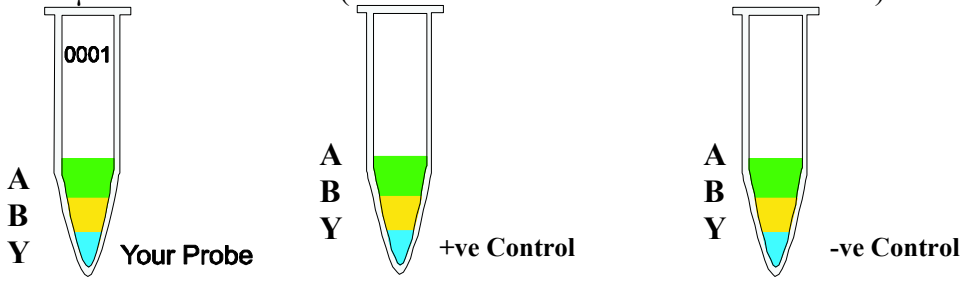
3. Add 1µl of tube Y to each tube.



4. Add 10µl of B to each micro tube. Avoid to touch the wall of the micro tubes.

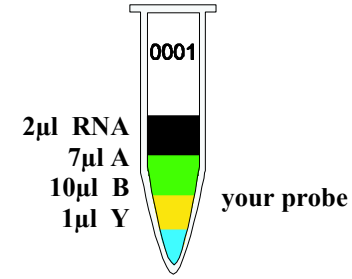


5. Add 7µl of A to each tube (avoid to touch the wall of the microtubes).

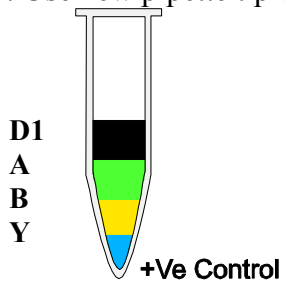


TIP: to save time and money, you can calculate how much chemicals you need to run the test. You want to run 10 test, i.e. you need 10 x 7µl of A = 70µl of A + 10 x 10µl of B = 100µl of B + 10 x 1µl of Y = 10µl of Y = 180µl in total. From this, 18µl can be added to each microtube.

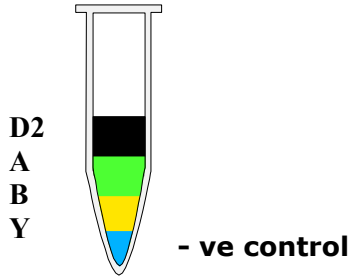
6. Add 2µl of your RNA with pipette tip with filter to each micro tube according to your label except +Ve and -Ve (Avoid touching the wall). **Use every time a new pipette tip** (for each sample)! Mix it.



7. Use new pipette tip with filter. Add 2µl of D1. Use a new pipette tip. Mix it.

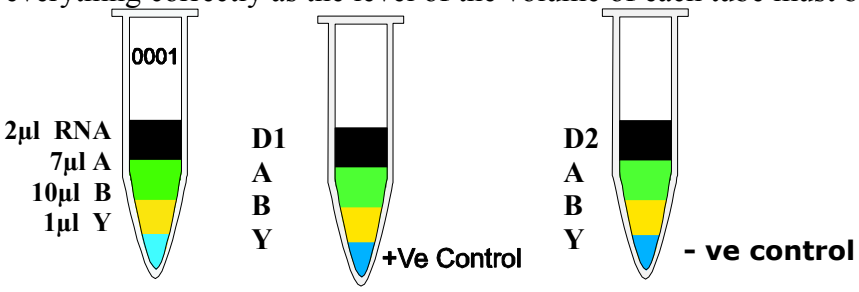


8. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control (avoid the wall). Mix it.



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. 3600 seconds at 42°C
   600 seconds at 70°C
   300 seconds at 37°C
2. A. 60 seconds at 94°C
   B. 60 seconds at 53°C
   C. 60 seconds at 72°C
   } x 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.

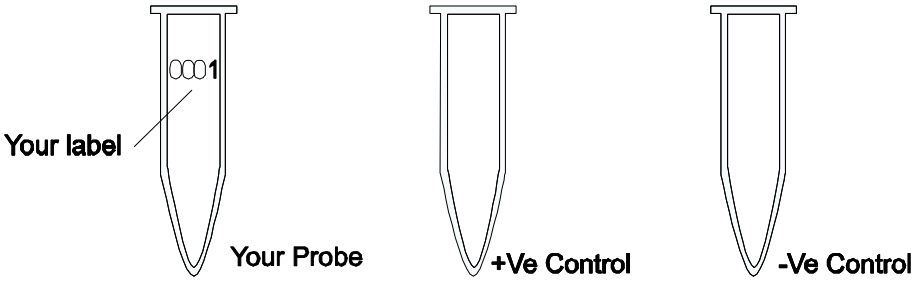
11. After step 10 is finished take out the microtubes.

To see Yellow fever virus, you can go directly to step gel electrophoreses (STEP B).

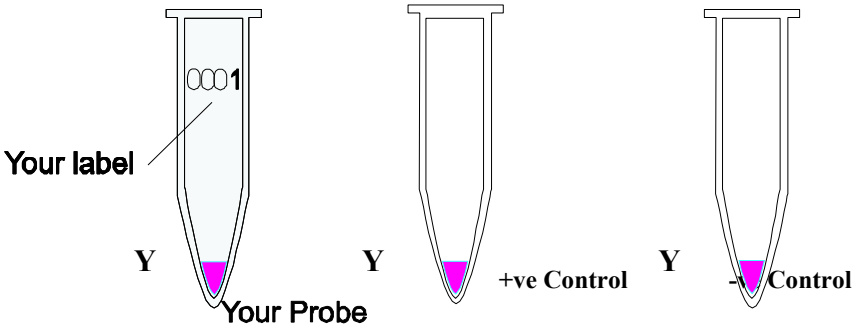
STEP B: in this step, you will detect only west nile virus.

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

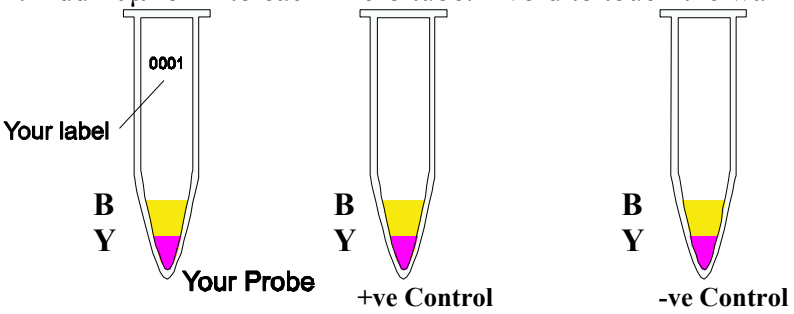
2. Mark your microtubes with a sample number and with +Ve Control and –Ve Control.



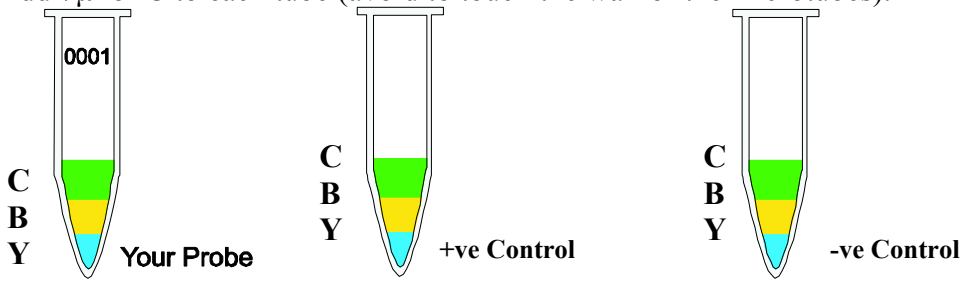
3. Add 1µl of tube Y to each tube.



4. Add 10µl of B to each micro tube. Avoid to touch the wall of the micro tubes.



5. Add 7µl of C to each tube (avoid to touch the wall of the microtubes).



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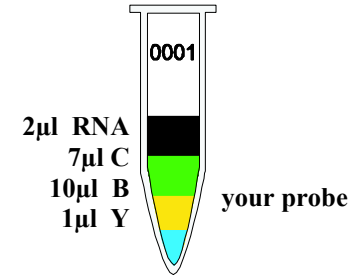
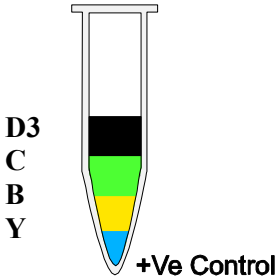
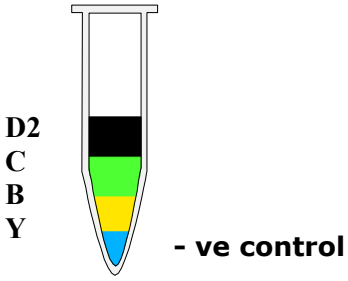


7. Use new pipette tip with filter. Add 2µl of D3. Use a new pipette tip. Mix it. **This is not included** to be made through user. If you do not have it, please run the assay without it.



8. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control (avoid the wall). Mix it.



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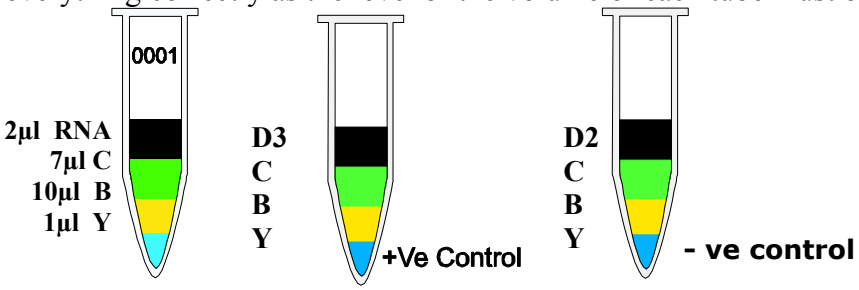

Now program your PCR machine as follows.

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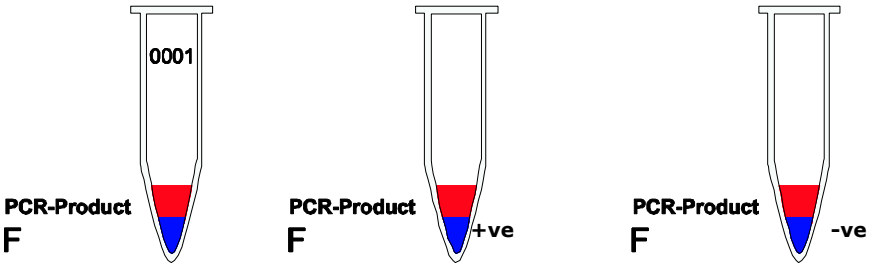
11. After step 10 is finished take out the microtubes.

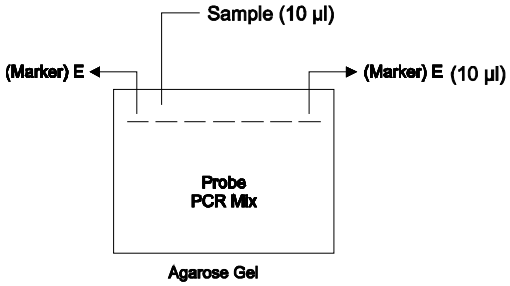
To see Yellow fever virus, you can go directly to step gel electrophoreses (STEP B).

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 **50 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 positive control and no band in negative control.
    The results are given on extra page.

Recommendation: gene sequencing is highly recommended for positive samples to be reconfirmed!

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: 19-12-2011** **v1.0**	**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**

K410: West Nile virus - RESULTS

1. In PCR product of Part A, you will see the following bands:

   675 band =======> Yellow fever (control cDNA): *this is included as control in our kit.*
   673 and 751 band => Japanese Encephalitis
   760 and 834 band => West Nile virus
   541 and 628 band => D2 (Dengue)
   552 and 607 band => D3 (Dengue)
   471 and 560 band => D4 (Dengue)

2. In PCR product of Part B, you will have a band of **229 bp**. This is specific for WNV. If you have WNV virus, please include this as control.