Lot-No.

Ref. FR193

MANUAL – Real time

Expiry date: 1 year

STORE AT -20°C

100 Tests (Ready to use kit)

HBV genotyping (Real time)

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

Principle and use

This amplification kit has been manufactured by *Genekam Biotechnology AG*, Germany to detect *HBV genotying (A, B, C, D, E, F, G and H)* in real time PCR. The assay is divided in two parts: first step will detect A, B, C and D. 2nd part will detect E, F, G and H. Both parts can be run at the same time in a machine or after one and other. Part one will be detected through Tube A and Part 2 will be detected through Tube C. Both parts with tube A and C must be run in separate tubes.

Warning: Multiplex assays need a real time machine with 4 channels or more. If you are using a machine with less channels, the results can be read only in used channels.

Real time PCR is based on fluorogenic dyes. In this kit, there are 4 probes / dyes, hence you have to program them in your machine, each probe indicates the presence of one specific virus:

Part one (Tube A):
First Probe: they are 6-Carboxy tetramethyl rhodamine (quencher) and Carboxy-fluorescein (reporter; also called FAM in some machines). Up to 40 Ct should be taken positive. Value between 40-45 Ct should be taken as marginal positive (doubtful). It indicates the presence of HBV genpotype A.

Second probe: they are 6-Carboxy tetramethyl rhodamine (quencher) and HEX (reporter, it is available as VIC in some machines). Up to 40 Ct should be taken positive. Value between 40-45 Ct should be taken as marginal positive (doubtful). It indicates the presence of HBV type B.

Third probe: they are 6-Carboxy tetramethyl rhodamine (quencher) and ROX (reporter, it is available as ROX in some machines). Up to 40 Ct should be taken positive. Value between 40-45 Ct should be taken as marginal positive (doubtful). It indicates the presence of HBV type C.

Fourth probe: they are 6-Carboxy tetramethyl rhodamine (quencher) and Cy5 (reporter, it is available as Cy5 in some machines). Up to 40 Ct should be taken positive. Value between 40-45 Ct should be taken as marginal positive (doubtful). It indicates the presence of HBV type D.

Part two (Tube C)

First Probe: they are 6-Carboxy tetramethyl rhodamine (quencher) and Carboxy-fluorescein (reporter; also called FAM in some machines). Up to 40 Ct should be taken positive. Value between 40-45 Ct should be taken as marginal positive (doubtful). It indicates the presence of HBV genpotype E.

Second probe: they are 6-Carboxy tetramethyl rhodamine (quencher) and HEX (reporter, it is available as VIC in some machines). Up to 40 Ct should be taken positive. Value between 40-45 Ct should be taken as marginal positive (doubtful). It indicates the presence of HBV type F and H.

Third probe: they are 6-Carboxy tetramethyl rhodamine (quencher) and ROX (reporter, it is available as ROX in some machines). Up to 40 Ct should be taken positive. Value between 40-45 Ct should be taken as marginal positive (doubtful). It indicates the presence of HBV type G.

Hint: Please make sure that you have realtime machine, which can detect all 4 probes in their corresponding channels. User has to select above said setting in the software of the realtime machine.

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

Safety precautions should be taken. Always clean your hands before the test use and clean the hands after the test. Wash your face after the test, if possible. Disinfect your working place.

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 (2 tubes)

Tube C (2 Tubes)

- Positive (+Ve) Control (tube D1) (1 tube)
- Negative (-Ve) Control (tube D2) (1 tube)

Please check them before you start. Please store them at -20°C and dark.

Equipment needed:

- Laboratory centrifuge
- Microtubes (0.2ml)
- Pipettes with and without filter (20µl, 5µl & 1µl)
- Pipettes (quality pipettes)
- Paper and pen
- Vortexer
- 96 well microplates for PCR
- Real time machine

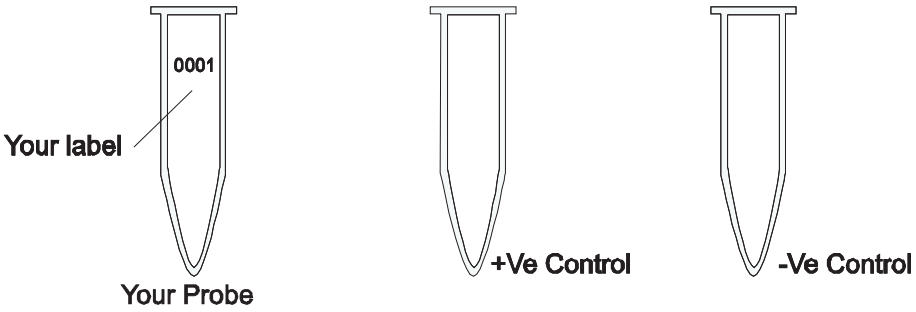
Procedure:

After your DNA isolation is completed. (Kindly use good quality isolation method).
Please go to PCR step

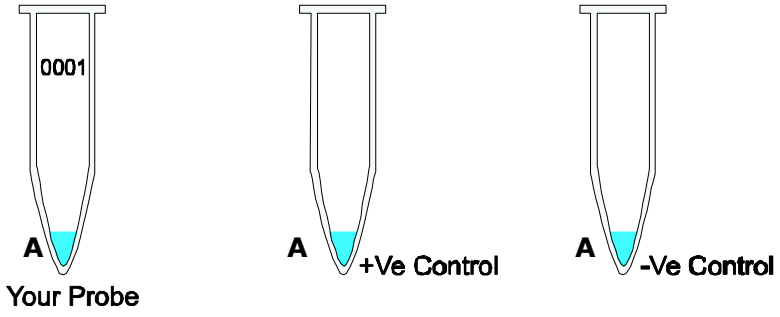
STEP A

1. Kindly thaw **one tube** each: A, B, C, D1 and D2. 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 it is not in use, store at -20°C.

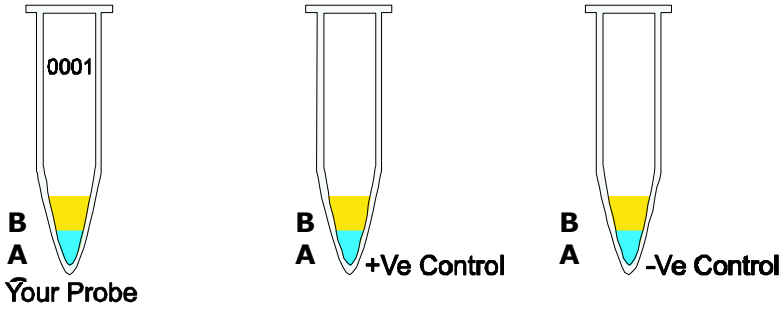
2. Mark your microtubes with a sample number and with +Ve Control and –Ve Control. **User will work first with Tube A as part one to detect the genotypes A, B, C and D and 2nd part will be tube C, hence it can be done at the same or in second part. *Both parts with tube A and C must be run in separate tubes!***



3. Thaw tube A. Add 8µl of tube A to each tube. One can also use 96 microwell plate.

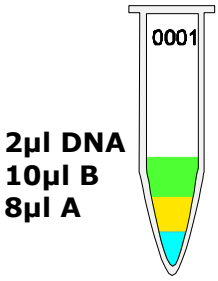


4. Add 10µl of B to each microtube. Avoid to touch the wall of the microtubes.

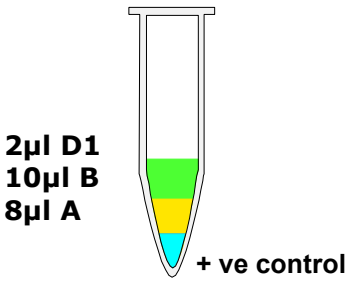


5. TIP: you can mix 8µl of A or C + 10µl of B together in one tube (it will be a total volume of 180µl for 10 reactions). From this one can take 18µl and distribute in each tube. In this way one can save the hardware and time.

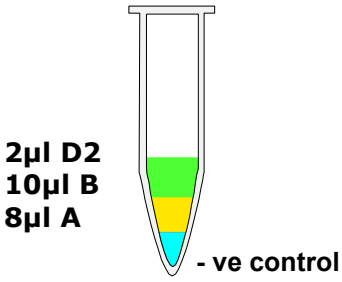
6. Add 2µl of your DNA template (DNA isolated from samples) with pipette tip with filter to each microtube according to your label except +Ve and –Ve (Avoid touching the wall). **Use everytime a new pipette tip** (for each sample)! Mix it.



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.

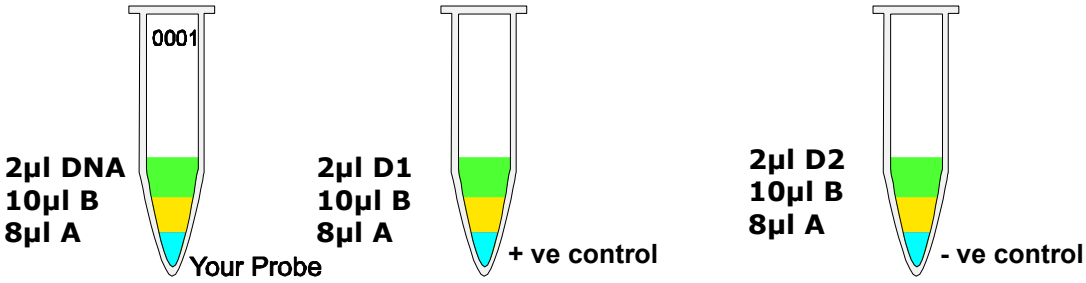


8. Use a new pipette tip. Add 2µl of –Ve (Tube D2) to –Ve Control (avoid to touch the wall). Mix it.



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

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.



You must use quencher and reporter dye to setup your software (see FAQ) and run the following program:

15 seconds at 95°C
60 seconds at 60°C } x 45 cycles

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

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

STEP B

Once the program will be finished one can see the graphics. The negative control should run along with the bottom and positive control must give a curve in the software graphics. Use your software to analyse the results.

Results with Tube A will indicate whether the sample has A, B, C and D genotypes.
Results with Tube C will indicate whether the sample has E, F, G and H genotypes.

If you should find any mistakes, please let us know. Thank you.

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

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: 13-02-2019
v1.0

FAQ:

1) Q: I cannot find quencher and reporter dye in my software:

A: Many software has got the words: FAM (as reporter) and TAM (as quencher). Therefore select both in your software.
If your machines has only one word (for some machines only use the word FAM) you should select this one.