Lot-No.

Ref. FR046

MANUAL – Real time one step

Expiry date: 1 year

STORE AT -20°C

100 Tests (Ready to use kit)

BOVINE VIRAL DIARRHOEA VIRUS (BVDV) – one step (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 *Microboss Hightech GmbH*, Germany to detect *Bovine viral diarrhoea virus (BVDV)*. ***This is an absolute quantification assay***. It is to differentiate between BVDV (1&2), too.

Real time PCR is based on fluorogenic dyes. In our kit we use **4 dyes:**

1) There will be Yakima Yellow (YYE; filter to be used is VIC) as reporter and Blackhole quencher (BHQ-1) as Quencher. Up to 36 Ct should be taken positive-. Value between 36-40 Ct should be taken as marginal positive (doubtful). **This will detect BVDV 2.**

2) Then there are Blackhole quencher (BHQ-1) and Carboxy-fluorescein (reporter). Up to 36 Ct should be taken positive. Value between 36-40 Ct should be taken as marginal positive (doubtful). **This will detect BVDV 1.**

This kit needs RNA which can be isolated from blood, serum, faeces, respiratory probes, aborted material, tissue, cell culture, vaccine 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 performing the assay.***

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 Y (1 tube)
- positive (+ve) control (D1): **to be stored at -20°C** (1 tube)
- Negative (-Ve) Control (tube D2) (1 tube)

Please check them before you start. **Please keep all tube away from light.**

Equipments needed:

- Realtime PCR thermocycler
- Laboratory centrifuge
- microtubes (0.2ml)
- sterile Pipette-tips with and without filter (20µl, 5µl & 1µl)
- Pipettes (quality pipettes)
- Paper and pen
- Microtube
- Ice
- Vortexer
- 96 well PCR plate

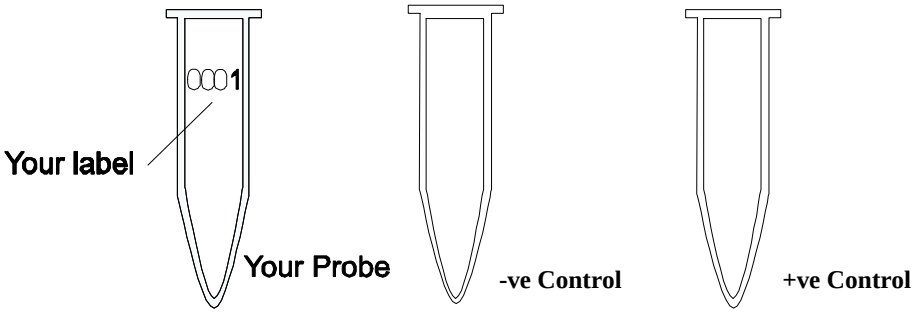
Procedure:

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

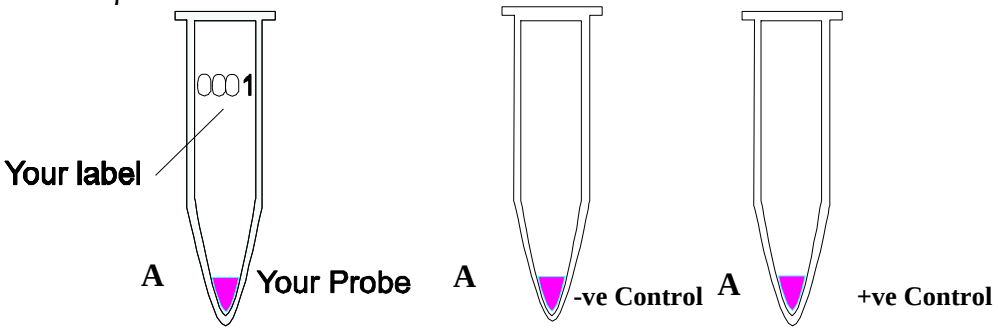
STEP A

1. Kindly thaw one tube each: A, B, Y, D1 and D2. After thawing, kindly put the tubes at 4°C (as it is better). Store this at -20°C, if it is not in use.

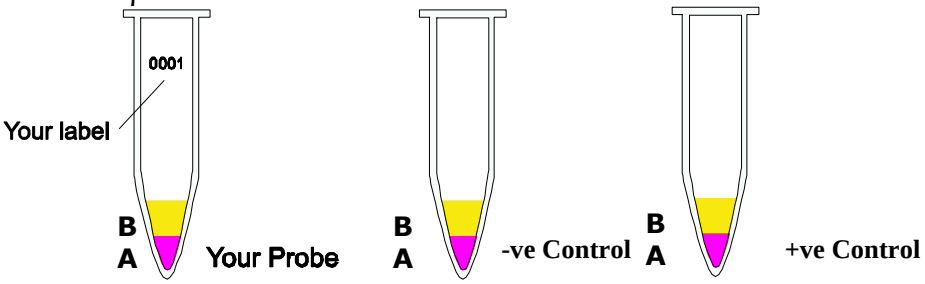
2. Mark your microtubes with a sample number, +ve Control and –ve Control. You can use 96 well microplate instead of tubes.



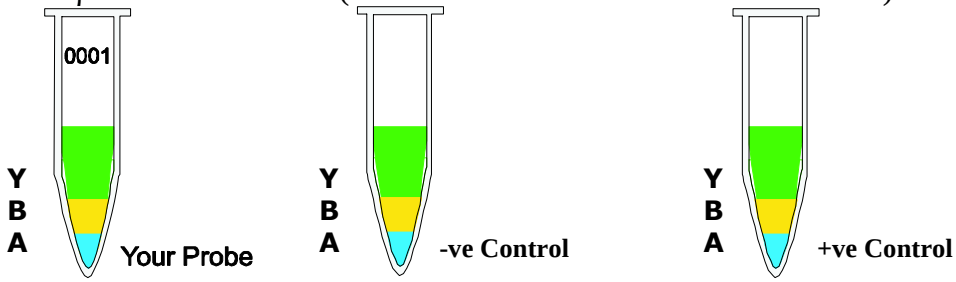
3. Add 7µl of tube A to each tube.



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

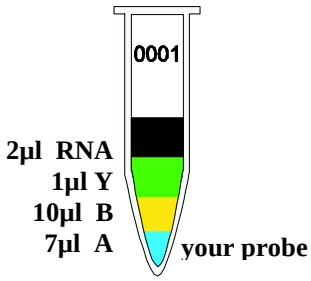


6. Add 1µl of Y to each tube (avoid to touch the wall of the micro tubes).

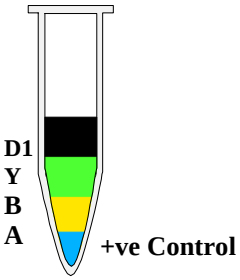


TIP: Add 7µl A + 10µl B + + 1µl Y = 18µl per reaction. In case you want to run 10 reactions i.e. you need total 180µl, therefore you should mix 70µl of A + 100µl of B + 10µl of Y = 180µl from which you can take 18µl and add to each tube. This way you save time and hardware.

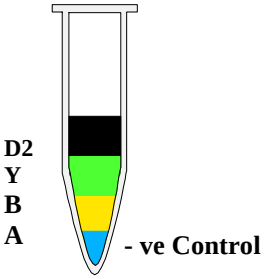
7. Add 2µl of your RNA with sterile pipette-tip with filter to each micro tube according to your label except +Ve and -Ve (avoid touching the wall). **Use every time a new pipette tip** (for each sample) ! Mix it.



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

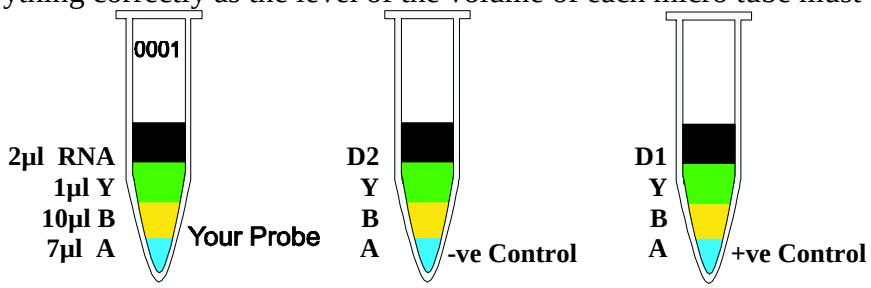


9. Use a new pipette tip. Add 2µl of –Ve (tube D2) to –Ve Control (don't touch the wall).Mix it.



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 micro tube must be almost the same.



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

1. 60 minutes at 42°C
   10 minutes at 70°C

2. 15 seconds at 95°C
   60 seconds at 60°C
   } x 40 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.

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.

How to interpret the results:

- If there are two curves in one well that it means the sample has got BVDV 1 and BVDV 2.
- If there is one curve in the well, kindly check the kind of the dye to see whether it is BDVD 1 or BVDV2.
- If there is no curve in the well , it is negative.

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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v1.1

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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.