

Instruction per step

Warnings

1. Switch on all devices.



2. Label the samples with numbers (1–8) and place them in order in a suitable rack.

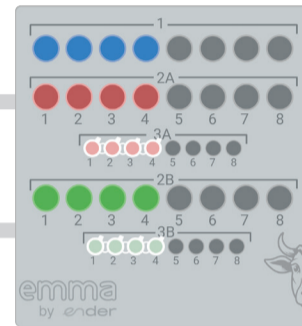


3. Start the «Real-time PCR system» soft-ware on the analysis Laptop and enter the relevant data for the analysis.



The length of the sample name is limited to 35 characters, and special characters are not permitted.

4. Prepare the required number of **tubes 1**, label them with the sample number and place them in the cooling block.



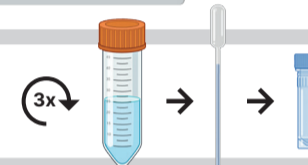
5. Prepare the required number of tubes of component **2A** and **2B** and place them in the appropriate place in the cooling block.

Shake the liquid downwards. Immediately return any unused tubes to the refrigerator.

6. Prepare the required number of tubes of component **3A** and **3B**, place them in the cooling block.

Shake the liquid downwards. Immediately return any unused tubes to the freezer.

7. Invert the samples 3 times and transfer ~0.5ml of each sample into the corresponding **tube 1**, using a new Pasteur pipette.



8. Homogenize all samples (40s, à 4350xg), then place the tube back in the cooling block.



9. Remove 10 µl of sample from **tube 1** and add it directly to tube **2A**. Mix up and down with the same pipette tip

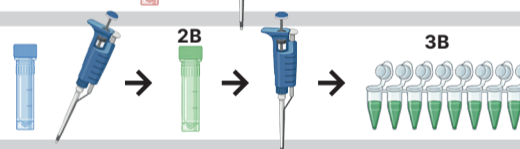


Remove the volume from the layer between the fat (top layer of the liquid phase) and the beads.

10. With the same pipette tip, take 10 µl and add it to tube **3A** without mixing. Close the lid.



11. Repeat steps 9–10 **with a new pipette tip**, for component **2B** et **3B** for the same sample.



12. Repeat steps 14–18 **with a new pipette tip for each other samples**.

13. Tap the strips lightly on the table and check that all the liquid is at the bottom of the tube.

Make sure that all the lids are tightly closed. **IMPORTANT!** This helps to ensure that the entire sample is mixed with the reagents.

14. Place the strips of components **3A** et **3B** in the thermocycleur according to the colour marking. Then press the lid of the device shut until it clicks into place.



IMPORTANT: The opening of the tubes always faces forwards (see picture). Add tubes from left to right.

15. Go back to the laptop and start the programm. After the run, the file is exported automatically.

START

16. When the device has performed the measurements, open the analysis software in the web browser at pqr.emma.vet and log in.

17. Import the file from the «lis» folder (in Quick Access) and click on «Upload and evaluate»

18. Export or print results as needed.
If the result of the sample is «Invalid», it should be repeated. To do this, add 10µl of the homogenised sample from tube 1 to component 4 «Magic tube». Then invert tube 4 so that the sample mixes well. Continue with tube 4 as described in step 9 until the end of the present protocol.

19. Switch off the devices, tidy up and clean the workplace.