

ddPCR Detection Method

Standard Operating Procedure (SOP)

Using the DQ24 automated digital PCR integrated system to establish a detection workflow, the specific operations cover four stages: system preparation, droplet generation, PCR amplification, and droplet fluorescence signal acquisition.

1. Preparation of Reagents

The total volume of the M.bovis digital PCR reaction system is 22 microliter, which includes: 11 microliters of $2 \times$ dPCR Probe Master Mix Plus, 1 microliter each of forward and reverse primers, 1 microliter of M.bovis DNA, and then add the pre-diluted probe at a concentration of 300 nanomolar to make up a total volume of 22 microliters.

2. Test Procedure

1. First, Press the power switch on the back of the instrument, after passing the self-test, the software automatically enter the main GUI.
2. Click **【New】** to create a new experiment.
3. Experiment name can be modified on the **【Properties】**, and select **Consumable Type/ Work Mode/Experiment Type/ Reagents Type**.
4. Click a well in the wells layout to display **【sample】** and **【target】**.
5. Define dPCR thermal parameters according to the reagent's instruction. The reaction procedure is as follows: keep the droplets stable

at 60 degrees Celsius for 5 minutes; then activate the enzyme at 95 degrees Celsius for 5 minutes, denature at 95 degrees Celsius for 10 seconds, and anneal/extend at 60 degrees Celsius for 30 seconds, for a total of 45 cycles; choose the Cy5.5 channel for droplet detection.

6. Check the Droplet generation oil level, Click **it** to open the front cover.

7. Prepare the consumables for the experiments based on the number of reactions required.

8. Load the Droplet tip Cassette into the sample holder, then place the Sample holder back into the instrument.

9. Load the 8 well Reaction Plate and the 8 well Seal Cover.

10. Load the **eight-tube strip** into the sample holder, and close the front cover **(remove the caps of eight-tube strip).**

11. The instrument automatically starts to check the consumables **(green indicates the consumables are loaded in the correct**

location, yellow indicates overloaded and red indicates lack of consumables). Front cover must be open to check the consumables if

there is any yellow and/or red hint **(the instrument will recheck the consumables after closing the front cover)**

12. Check the results.

13. Insert the USB disk, click the **[Export]** and send the chosen results to the USB disk.

3. Result judgment

1. Confirm threshold / Draw threshold. In Analyze Plot, select 1D dot

Plot to confirm and draw the threshold.

2. View Results Table / Export Results

(1) Nb Droplets, number of effective droplets

(2) Dilution, dilution factor, default is 1

(3) C (cp/μl), copy concentration. If the dilution factor is 1, the concentration is the number of copies per μL in the total system. If the dilution factor is not 1, it will be explained separately.

(4) Nb Pos, number of positive droplets in this channel