

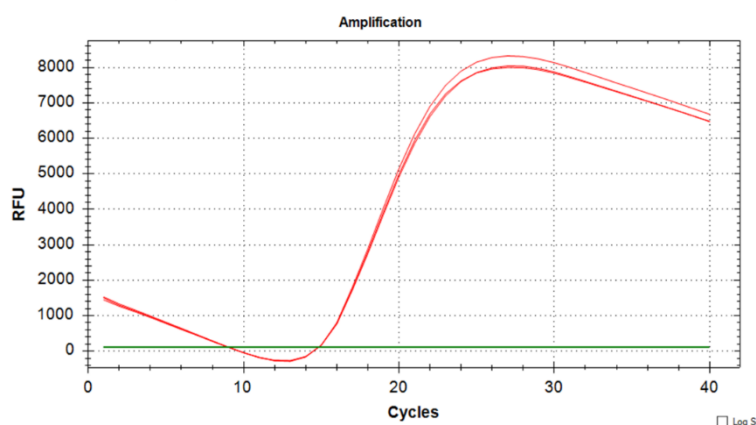
Troubleshooting for use of IdPath™ COVID-19 Real-Time RT-PCR Kit (Cat no. IP2000)

Listed below are some symptoms may appear in the detection of SARS-CoV-2 virus nucleic acid by IP2000 Kit. Possible causes and solutions are also provided for each symptoms.

1. Symptom : Poor signal or no signal in positive control (DNA) /internal control (RNA)

- Possible cause and solutions
 1. Exposure to hypochlorous acid water during RNA extraction will affect RNA stability. Please avoid using hypochlorous acid water during operation.
 2. The poor water quality of ddwater will affects fluorescence. It is recommended to use the ddwater included in the kit and avoid using other sources of ddwater.
 3. The long time preparation of RT-qPCR will decrease the fluorescence. The operation time from mixture preparation to inserting plate into PCR machine should not exceed over 2 hours.
 4. The lid of the 8-strip tube will reduce the fluorescence. Please use 96 well plate to prevent the lid from blocking the fluorescent light.
 5. The storage environment or operation method may be inappropriate. Check storage condition and follow the procedure of instruction for use strictly.

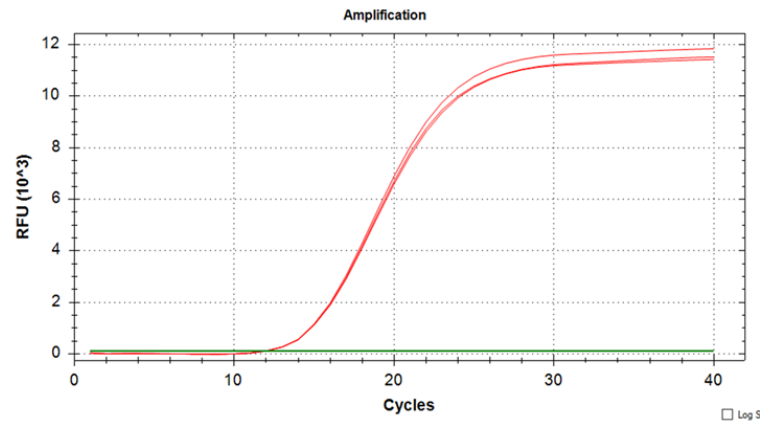
2. Symptom: The response curve is abnormal and rotates clockwise



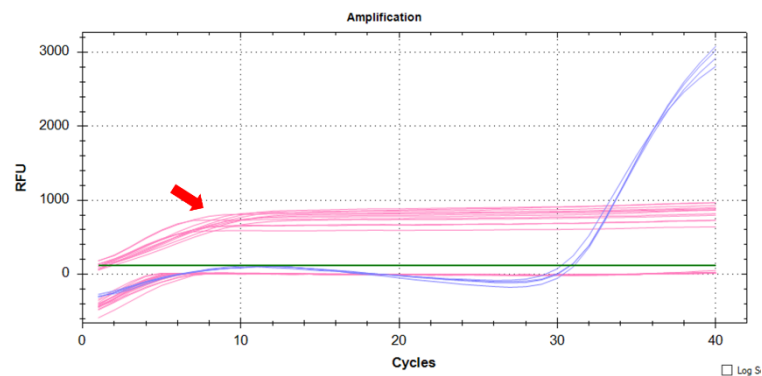
- Possible cause and solutions

The virus load is too high or the software analysis setting is not suitable.

Adjust the analysis settings. Set baseline to auto or adjust the cycle range of baseline where the curvature is horizontal, such as 6-10. The corrected curve will be as follows:



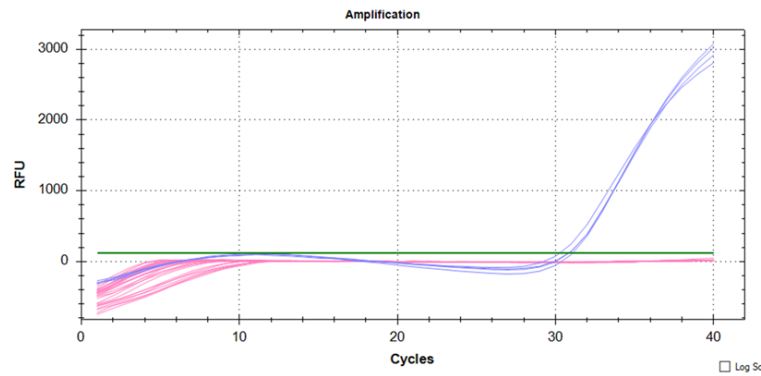
3. Symptom : The response curve drifts upward



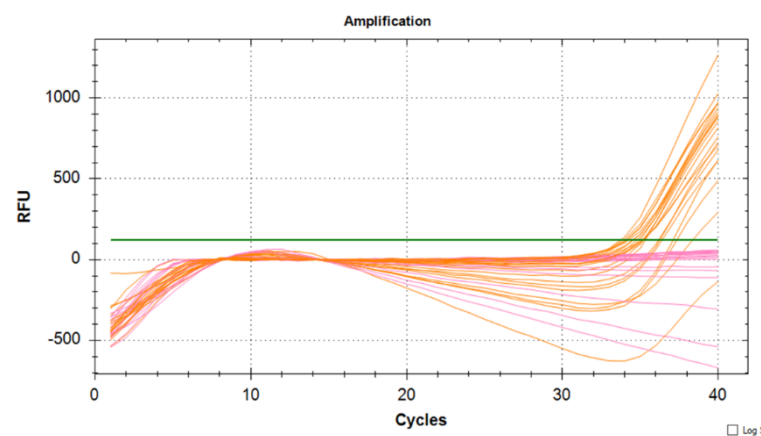
● Possible cause and solutions

This phenomenon may be related to the condensed water under the reaction plate and the baseline is not correctly set at 6-15. Please avoid placing 96 well plates on ice to prevent moisture under the plate. Use lens cleaning paper to carefully wipe the bottom of 96 well plates to make sure that no moisture remains.

Adjust the baseline settings and check the baseline is set at 6-15 may correct the curve. The corrected curve is shown as below. If the curve is still abnormal after adjustment, users may refer to the fourth symptom for further instructions.



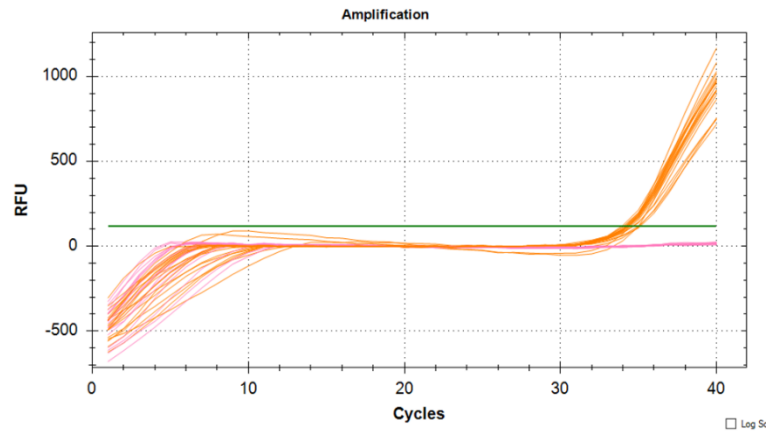
4. Symptom : The response curve is downward



- Possible cause and solutions

This phenomenon may be related to the condensed water under the PCR plate. The inconsistency of initiation point on the reaction curve may cause the response curve downward significantly. Please avoid placing 96 well plates on ice to prevent moisture under the plate. Use lens cleaning paper to carefully wipe the bottom of 96 well plates to make sure that no moisture remains.

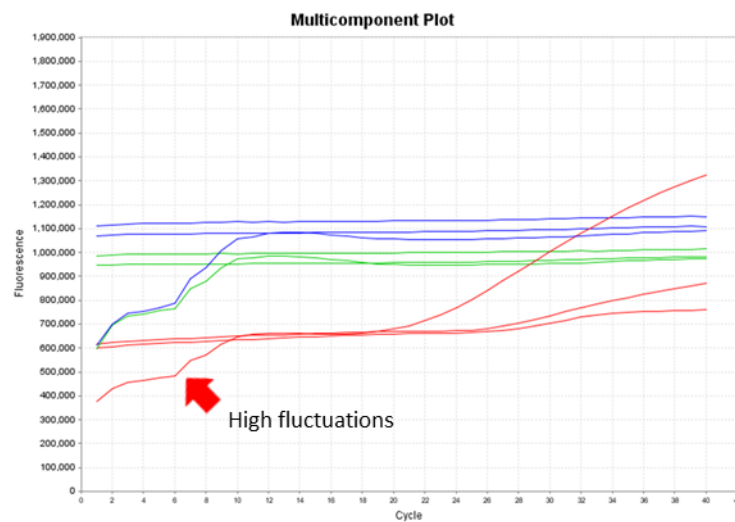
Adjust the analysis settings may correct the abnormal curve. Set baseline to auto and check the option "Apply fluorescence drift correction" in the baseline setting to correct the curve. The corrected curve may be as follows:



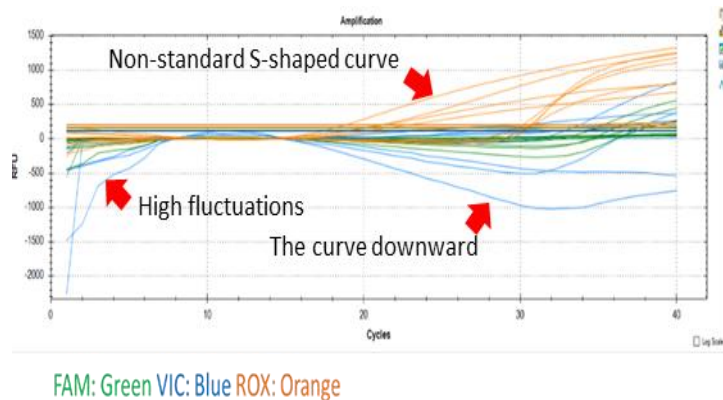
5. Symptom : The response curve appears messy

- (1) The response curve shows high fluctuations at the beginning.
- (2) Presents a non-standard S-shaped curve or the curve downward at the end.

Example 1



Example 2



- Possible cause and solution:
This phenomenon may be related to the presence of bubbles in the reaction. As dispensing liquid by manual pipette, press down the push injection button slowly and smoothly to the first stop but do not press down to the second stop to avoid bubble generation. Before the reaction on the machine, please centrifuge the 96 well PCR plate to reduce air bubbles.

6. Symptom : Signal in negative control

- Possible cause and solutions
Poor laboratory technique can result in contamination of PCR samples. Contamination will be evidenced by the presence of an amplification signal in negative control samples prepared with all reaction components except the DNA template.
 - (1) To minimize the possibility of contamination of PCR components by PCR product or other template, designate a work area exclusively for PCR assay setup.
 - (2) If necessary use a solution of 10% bleach instead of 75% ethanol to prepare the workstation area for PCR assay setup. The 10% bleach solution will hydrolyze, as well as dissolve, any residual DNA and RNA.
 - (3) When dispensing the sample, please directly move to the target position in a single direction and avoid passing over the NTC.
 - (4) In general, follow these practices to minimize the risk of sample contamination:
 - a. Wear gloves
 - b. Use screwcap tubes
 - c. Use aerosol-resistant filter tips
 - d. Use the ddwater included in the kit
 - e. Prepare a master mix with sufficient volume to prepare all replicate samples
 - f. Use the UV lamp to decontaminate the environment after the operation.