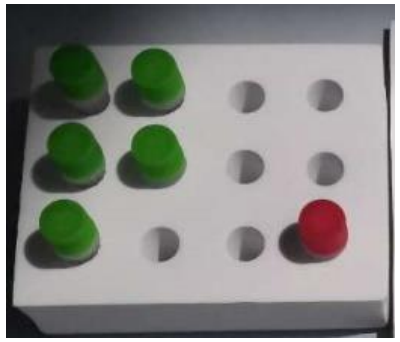
Corona Virus Disease 2019 (CoViD-19) Nucleic Acid Detection Kit (Real-Time PCR Method).

Experiment Process

Operation



Reagent preparation ①

Reaction solution preparation ②

Load sample 20μl/well ③

Nasopharyngeal swab, sputum, alveolar lavage fluid sample preparation ④

RNA extraction ⑤

RNA

Load sample 5μl/well ⑥

The octet is first loaded with the reaction solution, and followed by the RNA sample addition

Put on the thermocycler ⑦

1. Reagent Preparation

Remove the kit from the refrigerator below -15℃ and put at room temperature (20-25℃) for thawing.

After complete thawing, shake, mix thoroughly and centrifuge at low speed for 10s.

Calculate the number of reactions required for the experiment to be carried out.

According to the preparation method of the reaction system on the table (left), the reagents used are sequentially sub-packed into the PCR reaction tubes.

Loading sequence	Component	Volume per test	it is recommended to prepare 13 samples for 10 tests
1	Reaction solution	17μL	321μL
2	Mixed enzyme solution	3μL	39μL

Note: It is recommended to prepare 3 additional reaction system for each test, which are used to detect negative positive control substances and as a reserve for the loss during preparation.

2. RNA Extraction

- It is recommended to use the commercially available nucleic acid extraction kits to extract sample RNA for PCR detection and follow the operation steps of the extraction kit instructions.

- The negative control and positive control in this kit are all involved in extraction.

3. Sample Loading

1). Take out the prepared reagents in the reagent preparation area and centrifuge at low speed for 10s.

2). Add the RNA to be tested, the positive control and the negative control to the PCR reaction tube, respectively, in an amount of 5μL/well.

3). Cover the PCR reaction tube, record the template loading sequence, and centrifuge at low speed for 10s.

4). Transfer the PCR reaction tube to the nucleic acid amplification area for processing.

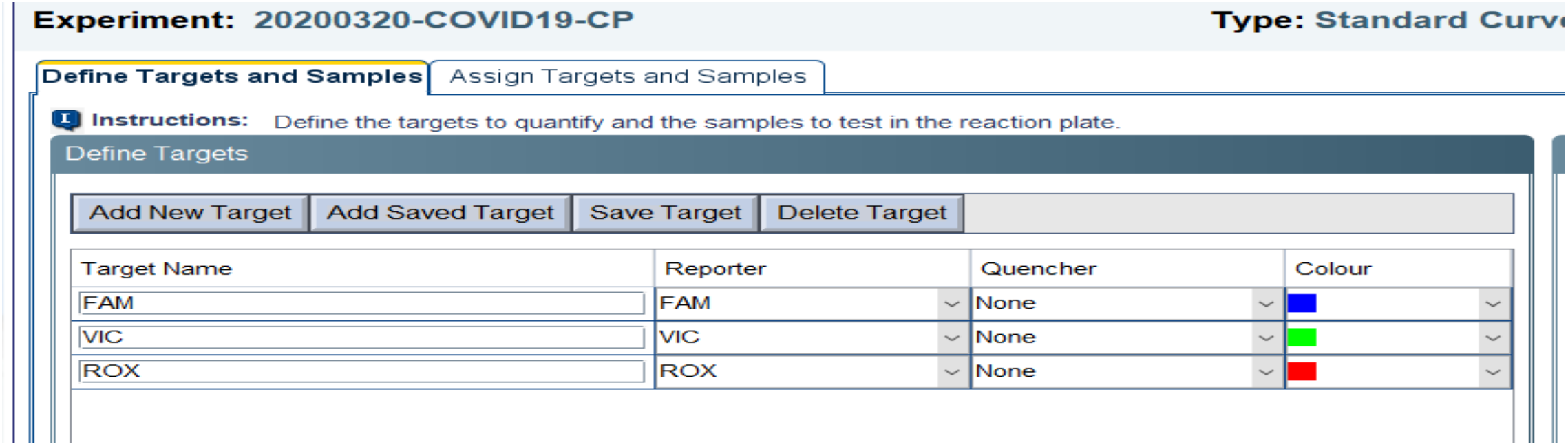
Note: *Avoid contamination during RNA sample extraction and loading. If the extracted RNA template cannot be detected immediately, it is recommended to store it below -70°C.*

4. Processing On The Thermocycler

1. Please follow the instructions of the Real-Time PCR detection System for test operation (Here ABI 7500/7900).

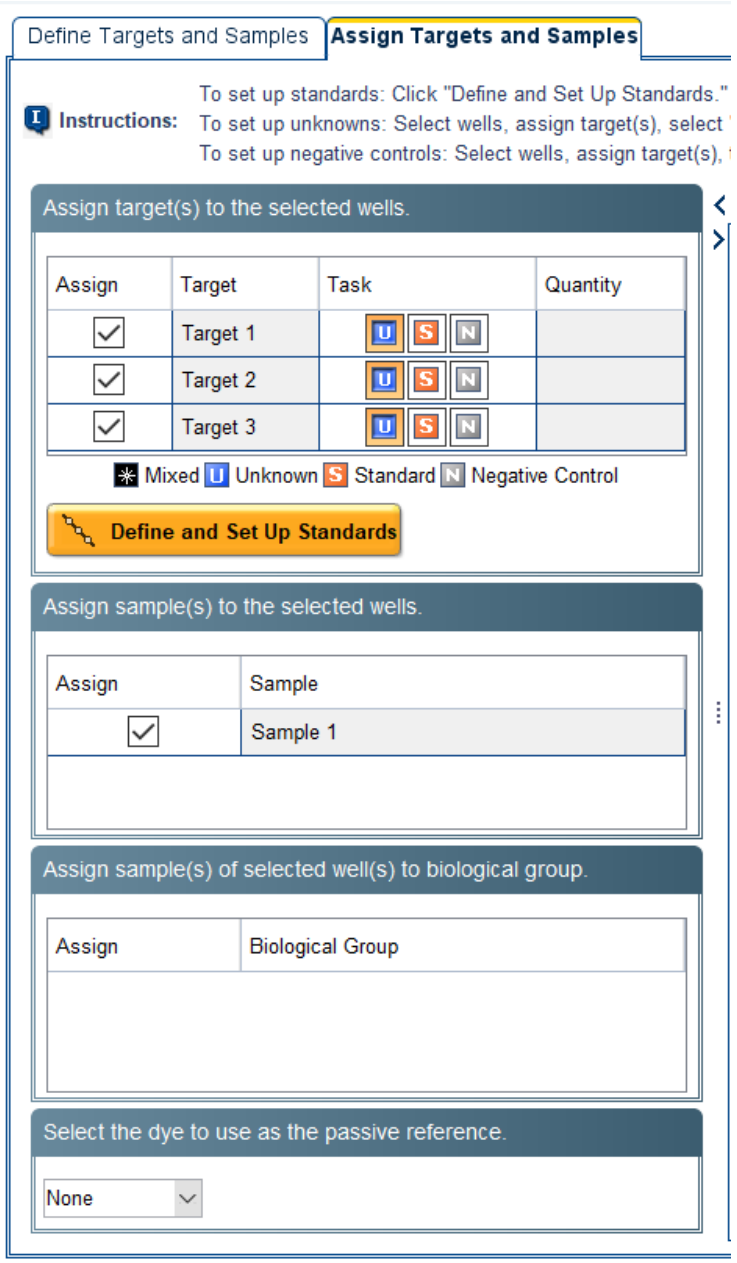
2. Setup → Experiment Properties [Fill in] Experiment Name, [Select one by one] 7500 96well)， Quantitation-Standard Curve, Taqman@Reagent and Standard (~2 hours to complete a run) [mode].

3. Plate Setup → Define Targets and Samples [Edit] Target Name (For) FAM、VIC (And) ROX [And select accordingly] Reporter (Fluorescence)， Quencher [Quenching groups and colors].



4. Processing On The Thermocycler

4. Plate Setup → Assign Targets and Samples (Select test samples, click FAM, VIC and Cy5, and Sample 1, Passive Reference select None).



4. Processing On The Thermocycler

5. Run Method

One step PCR amplification standard procedure：

Stage 1: Reverse transcription, 1 cycle,

50°C for 15 minutes;

Stage 2: Pre-reaction, 1 cycle

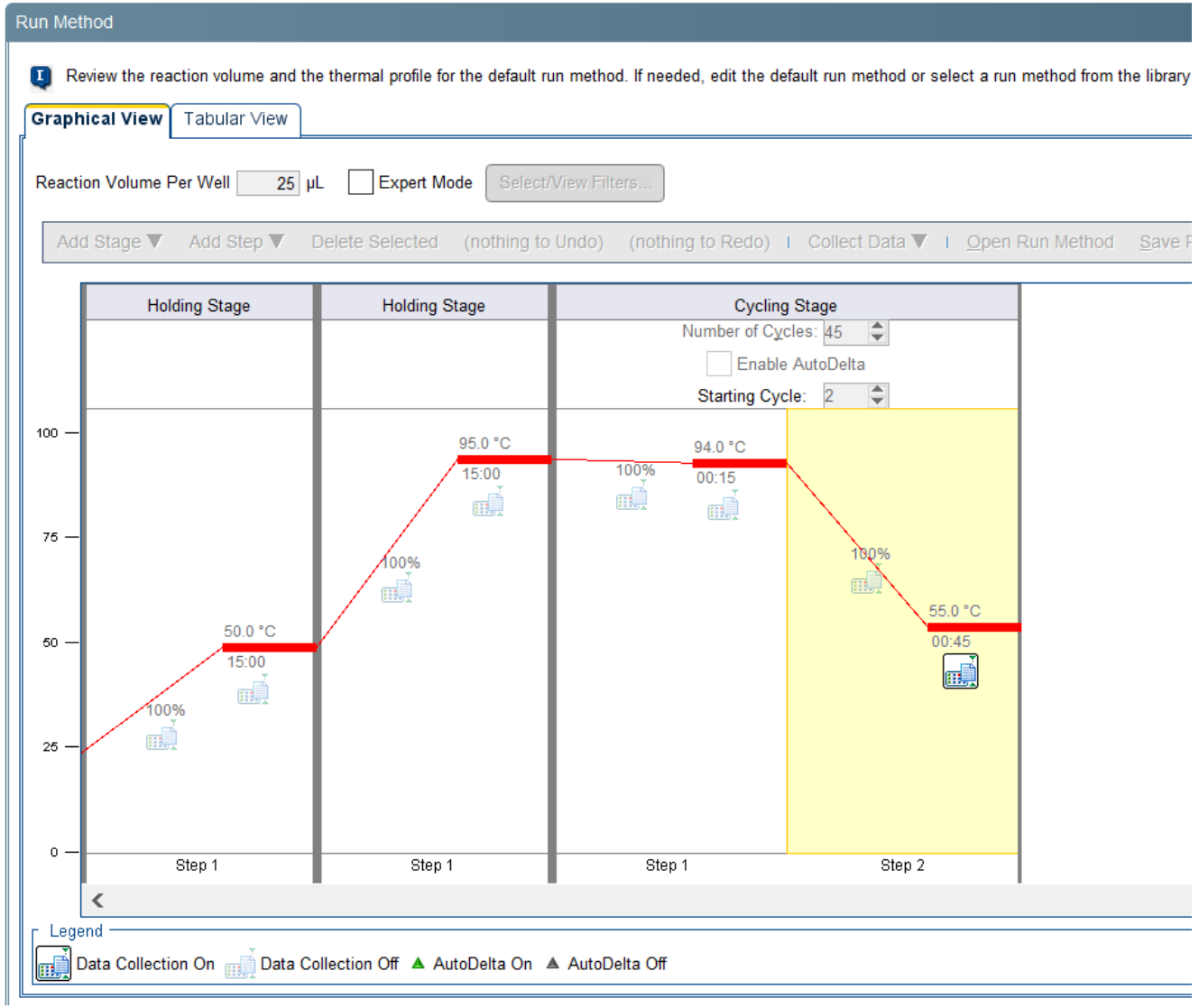
95°C for 15 minutes

Stage 3: PCR reaction, 45 cycles.

94°C 15 seconds, 55°C 45 seconds；

Collect FAM, VIC and Cy5 signals.

6. Save the file and run the experiment.



5. Results

Reading of the experiment results: Select ΔRn VS Cycle and Linear mode for data analysis; set the appropriate threshold, select Auto Baseline, view the fluorescence curve, and obtain the fluorescence quantitative curve and Ct value in the quantitative PCR instrument.

5. Results

1. Kit reliability：

a). Positive control: FAM and VIC have obvious amplification curves, Ct value ≤32, Cy5 signal with or without amplification curve.

b). Negative control: FAM and VIC signals present no amplification curves, Cy5 signal has an obvious amplification curve, and the Ct value is ≤32.

c). The above requirements must be met in the same experiment at the same time, otherwise, the experiment is invalid and needs to be repeated.

5. Results

2. Sample results reading:

a). According to the following table, determine the detection results of the novel coronavirus:

	Test results		Test results and interpretation
	N gene (FAM channel)	ORF1ab (VIC channel)	
a	+	-	Suspected positive, repeat test
b	-	+	Suspected positive, repeat test
c	+	+	Positive
d	-	-	Negative

5. Results

2. Sample results reading:

b). Samples with positive single target detection items a and b need to be repeatedly sampled and tested. If the single target is still positive, the sample test result is positive.

c). If the negative standard Cy5 has no amplification curve or Ct> 40, the tested sample result is invalid. The cause should be found and eliminated, and the sample experiment repeated.

Note: *A negative PCR test cannot clinically exclude the possibility of COVID-19.*

6. Results Interpretation

- Negative control and positive control shall be tested in each experiment, and the test results can be determined only when the control meets the quality control requirements.

- When the FAM and VIC test channels are positive, the Cy5 channel result may be negative due to system competition.

- When the internal standard result is negative, if the FAM and VIC test signals of the test sample are also negative, the test result of the sample is invalid, and the cause should be found and eliminated, and the tested sample experiment repeated.

[Nota Bene - Applicable Instruments]

This kit is suitable for ABI, Applied Biosystems, Bio-Rad, Agilent (MX-3000), MGITech, LighCycler 480II Roche, SLAN thermocycler series. If your used thermocycler brand is not mentioned, please contact our Technical Department at ask@oacp.it for assistance.

OaCP IE LTD

PHOENIX HOUSE, MONAHAN ROAD, CORK, IE

WWW.OACP.IT

ASK@OACP.IT

In partnership with:
Wuhan HealthCare Biotechnology Co., Ltd.
No. 666, Gaoxin Boulevard, Optics Valley Biolake, Building B6, 4/5F.
Wuhan, Hubei, 430075, PR of China.