LyteStar 2019-nCoV RT-PCR Kit 2.0 Workflow

Kit Components



What is in the kit?

2x Master A (yellow cap)4x Master B (blue cap)1x Internal Control (green cap)1x Positive Control (red cap)1x Negative Control (clear cap)

1x Master A + 2x Master B = 48 reactions



Master Mix Setup for 48 reactions

1. Remove the following tubes from the box and let thaw completely:

1x Master A (yellow cap) 2x Master B (blue cap) 1x Internal Control (green cap) 1x Positive Control (red cap) 1x Negative Control (clear cap)



2. After all components have thawed completely, briefly vortex and centrifuge.



3. Prepare PCR Master Mix by adding both tubes Master B to Master A.

Set Pipette to **370** μ l and pipette the complete content of the 1st tube Master B (blue cap) into Master A (yellow cap).

Repeat the process with the 2^{nd} tube of Master B.



4. Add Internal Control into PCR Master Mix.

Set Pipet to $\mathbf{27} \ \mu l$

and pipette 27 $\mu l\,$ of the Internal Control (green cap) into the PCR Master Mix.



5. Briefly vortex and centrifuge the PCR Master Mix.



PCR reactions Setup

6. Pipette 20 μl of PCR Master Mix into each PCR tube.

Set the Pipet to ${f 20}~\mu l$

and add 20 μl of PCR Master Mix to each required Mic PCR tube.



7. Add the Negative Control

Set the Pipet to $\boldsymbol{5}\,\mu l$

and add 5 μl of PCR grade water to the appropriate PCR tubes for NTC.

Make sure to use a fresh pipet tip for each Negative Control.



8. Add the Samples

Add 5 μl of each sample to the appropriate PCR tubes.

Make sure to use a fresh pipet tip for each sample.



9. Add the Positive Control

Add 5 μ l of Positive Control to the appropriate PCR tubes.

Make sure to use a fresh pipet tip.



10. Close the PCR tubes

Tightly close the PCR tubes with the appropriate caps.

For Mic PCR tubes, use the provided capping tool to fit the caps properly.

Only for ultraSMBS24, add one drop of PCR mineral oil into each PCR tube before closing the tube.

11. Load the PCR cycler

Open the qPCR cycler and remove the tube clamp.

Starting from **Position 1**, place the PCR tubes in the qPCR cycler. Fill the unused wells with PCR tubes that are pre-loaded with 25 μ l of water.

Place the tube clamp back on and close the lid of the instrument.





Programming the ultraSBMS24





Programming the ultraSBMS24 real-time PCR instrument



Set the Hold stage Step 1-1 temperature and time as 50 degrees for 10 minutes (Reverse Transcription step)

Set the Hold stage Step 1-2 temperature and time as 95 degrees for 2 minutes (Initial Activation step)



4.

Enter the PCR Stage as 45

Set the PCR stage Step 2-1 temperature and time as 95 degrees for 5 seconds (Denaturation step)

Set the PCR stage Step 2-2 temperature and time as 55 degrees for 30 seconds (Annealing/Extension step)

5.

Ensure that *Lighting On* is set on PCR stage Step 2-2

Set Volume (ul) as 50

Click SAVE

6.

Enter *ADT COVID PCR Test* as the Template Name

Click ENSURE to save the template

Once the run profile has been saved as a template you do not need to repeat the programming of the ultraSBMS24 cycler but can simply open the template from the *Load Template* on the main interface for any new PCR runs.











interface
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9.

To choose the template, click on *ADT COVID PCR Test*.

Operator	Detect name	Time	Detect type	MANAGE
user	ADT COVID PCR Test	2022/07/01/ 09:52:48	QPCR	
user	Direct nCoV 2.0	2022/06/10/ 10:06:21	QPCR	
user	MEDsan ultraSBMS SARS-CoV-2	2022/01/17/ 15:19:56	QPCR	
user	ultraSBMS SARS-CoV-2-I	2021/11/26/ 16:30:01	QPCR	
user	ultraSBMS SARS-CoV-2-II	2021/11/26/ 16:30:00	QPCR	

10.

Click *NEXT* to enter the Run Conditions Interface

Detect name:	ADT COVID PCR Test	
Username:	user	
		<-BACK
Detect type:	QPCR	NEXT>>

7.

main interface.

Click the HOME button to return to the

Click LOAD TEMPLATE on the main

Enter Volume (ul) as 50

Click NEXT to enter the Sample Setup interface



12.

Select the sample well position to be tested and click EDITOR to edit the sample information of this well.

1	2	3	4	5	EDITOR
6	7	8	9	10	
11	12	13	14	15	
16	17	18	19	20	<-BACK
21	22	23	24	ALL	Run

Sample Setup

13.

In the Sample Setup box, enter the Sample name and select the following Fluorescent dye/Reagent:

- FAM/SYBR •
- VIC/HEX
- CY5 •

For each Fluorescent dye/Reagent selected, choose the appropriate Task:

Sample – Unknown •

FAM/SYBR

VIC/HEX

ROX

CY5

- Positive Control (PC) Standard
- No Template Ctrl (NTC) Negative •

Click ENSURE after each sample setup is complete



Check to ensure all sample wells are labelled correctly and the correct fluorescent dyes are selected.

Click NEXT

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	EDITOR
FAM/SYBR	FAW/SYBR	FAM/SYBR	FAM/SYBR	FAWSYBR	
VIC/HEX	VIC/HEX	VIC/HEX	VIC/HEX	VIC/HEX	
CY5	CY5	CY5	CY5	CY5	
1	2	3	4	5	
Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	
FAMSYBR	FAM/SYBR	FAM/SYBR	FAW/SYBR	FAM/SYBR	
VIC/HEX	VIC/HEX	VIC/HEX	VIC/HEX	VIC/HEX	
CY5	CYS	CY5	CY5	CY5	
6	7	8	9	10	
Sample 11	Sample 12	Sample 13	Sample 14	Sample 15	
FAWSYBR	FAM/SYBR	FAM/SYBR	FAWSYBR	FAWSYBR	
VIC/HEX	VIC/HEX	VIC/HEX	VIC/HEX	VIC/HEX	
CY5	CY5	CYS	CY5	CY5	
11	12	13	14	15	
Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	< <back< td=""></back<>
FAWSYBR	FAM/SYBR	FAM/SYBR	FAWSYBR	FAM/SYBR	
VIC/HEX	VIC/HEX	VIC/HEX	VIC/HEX	VIC/HEX	
CY5	CY5	CY5	CY5	CY5	
16	17	18	19	20	
PC FAM/SYBR	NTC FAM/SYBR	NTC FAM/SYBR	NTC FAM/SYBR		NEXT>>
CY5 21	VIC/HEX CY5 22	CY5 23	CY5 24	ALL	

Calibration curve OFF Curve display						DETERMINE
	1	2	3	4	5	
	6	7	8	9	10	
	11	12	13	14	15	
						< <back< td=""></back<>
	16	17	18	19	20	
0 5 10 15 20 25 30 35 40 45	21	22	23	24	ALL	🕞 Run

15.

Start the run by clicking the *RUN* button.

16.

In this interface, the progress and completion of the test are displayed.



Data Analysis the ultraSBMS24





Data Analysis

1.



2.

Click the Arrow to move to the Run Conditions interface

3.

Click the Arrow to move to the Run Curve interface

300 FAM/SYBR 270k On the Calibration curve bar turn the VIC/HEX Curve display ON to display the all the 240k 210k Cy5 180k 150k 17 13 120k VSYRR FAM/SYBR 90k VIC/HEX 18 19 20 60k 30



ALL

24

5.

Click on DETERMINE to enter the results analysis interface.

6.

This page displays the detailed information of the detection result, including the quantification cycle (Ct) value (4th column) and the interpretation of positive/negative results of the sample (6th column).

A positive result is indicated by a Ct value and a POSITIVE label in the table (e.g. Sample 1). A negative result is indicated by an absence of a Ct value and a NEGATIVE label in the table (e.g. Sample 17).

The "threshold" displays the positive threshold of intelligent interpretation that is set by the machine. This does not need to be altered.

To view each sample individually, click on the Sample of interest.

Determine							
Hole	Sample name	Channel	Ct	Conc.	Result	channel	
		FAM/SYBR	28.658		POSITIVE	FAM/SYBR	
1	Sample 1	VIC/HEX	24.557		POSITIVE	Threshold	
		CY5	33.637		POSITIVE	1672	
		FAM/SYBR	28.899		POSITIVE		
2	Sample 2	VIC/HEX	24.774		POSITIVE	SAVE	
		CY5	34.877		POSITIVE		
3 Sample 3		FAM/SYBR	29.228	-	POSITIVE		
	VIC/HEX	24.531		POSITIVE			
		CY5	33.900		POSITIVE		

Determine							
Hole	Sample name	Channel	Ct	Conc.	Result	channel	
		FAM/SYBR		-	NEGATIVE	FAM/SYBR	
17	Sample 17	VIC/HEX	24.847		POSITIVE	Threshold	
		CY5			NEGATIVE	1672	
		FAM/SYBR			NEGATIVE		
18	Sample 18	VIC/HEX	25.180		POSITIVE	SAVE	
		CY5			NEGATIVE		
	19 Sample 19	FAM/SYBR			NEGATIVE		
19		VIC/HEX	25.150		POSITIVE		
		CY5			NEGATIVE		

4.

collected data curves.

Analyse the PCR results for each individual sample and the validity of the overall PCR experiment as per the validation criteria set out in detail in the Instructions for Use; in brief,

> A. The No Template Control must always be negative in the FAM and CY5 channels, and positive in the HEX channel (Sample 22)

			Determine			X
Hole	Sample name	Channel	Ct	Conc.	Result	channel
		FAM/SYBR	20.486		POSITIVE	FAM/SYBR
21	PC	VIC/HEX	23.044		POSITIVE	Threshold
		CY5	27.427		POSITIVE	1672
		FAM/SYBR			NEGATIVE	
22	NTC	VIC/HEX	25.188		POSITIVE	SAVE
		CY5			NEGATIVE	
		FAM/SYBR			NEGATIVE	
23	NTC	VIC/HEX	25.063	-	POSITIVE	
		CY5			NEGATIVE	
		FAM/SYBR			NEGATIVE	
24	NTC	VIC/HEX	25.118		POSITIVE	
		Run Curve	•		×	<
	Hole:22		Sar	mple name:NTC		
FAM/SYBR				Cy5		
Ok						
Ok				/		
Ok				/		
Ok						
Ok				/		
Ok			/			
Ok						

 B. The Positive Control must be positive in all three channels (Sample 21)

			Determine			X
Hole	Sample name	Channel	Ct	Conc.	Result	channel
		FAM/SYBR	20.486		POSITIVE	FAM/SYBR
21	PC	VIC/HEX	23.044		POSITIVE	Threshold
		CY5	27.427		POSITIVE	1672
		FAM/SYBR			NEGATIVE	
22	NTC	VIC/HEX	25.188		POSITIVE	SAVE
		CY5			NEGATIVE	
		FAM/SYBR			NEGATIVE	
23	NTC	VIC/HEX	25.063		POSITIVE	
		CY5			NEGATIVE	
		FAM/SYBR			NEGATIVE	
24	NTC	VIC/HEX	25.118		POSITIVE	
		Run Curve	1		X	
	Hole:21		Sar	nple name:PC		

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 C. A positive sample is indicated by amplification in the FAM and Cy5 channels (e.g. Sample 1)

		1	Determine			X
Hole	Sample name	Channel	Ct	Conc.	Result	channel
		FAM/SYBR	28.658		POSITIVE	FAM/SYBR
1	Sample 1	VIC/HEX	24.557	-	POSITIVE	Threshold
		CY5	33.637		POSITIVE	1672
		FAM/SYBR	28.899		POSITIVE	
2	Sample 2	VIC/HEX	24.774		POSITIVE	SAVE
		CY5	34.877		POSITIVE	
		FAM/SYBR	29.228		POSITIVE	
3	Sample 3	VIC/HEX	24.531		POSITIVE	
		CY5	33.900		POSITIVE	
		Run Curv	re		×	
	Hole:1		S	ample name:Sample	e 1	



 A negative sample is indicated by an absence of signals in the FAM and CY5 channels, but must have amplification in the HEX (Internal Control) channel to rule out PCR inhibition. (e.g. Sample 17)

	X					
Hole	Sample name	Channel	Ct	Conc.	Result	channel
		FAM/SYBR			NEGATIVE	FAM/SYBR
17	Sample 17	VIC/HEX	24.847		POSITIVE	Fhreshold
		CY5			NEGATIVE	1672
		FAM/SYBR			NEGATIVE	
18	Sample 18	VIC/HEX	25.180		POSITIVE	SAVE
		CY5			NEGATIVE	
		FAM/SYBR			NEGATIVE	
19	Sample 19	VIC/HEX	25.150		POSITIVE	
		CY5			NEGATIVE	

